CHARACTERIZATION OF AND IMPROVEMENT IN THE NUTRITIONAL VALUE OF WHEAT MILLRUN FOR SWINE

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By

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ABSTRACT

Little information exists on the nutrient composition and value of wheat millrun as an opportunity feedstuff for swine. The nutritional value of millrun and ways to improve it were investigated in 4 studies. In Chapter 1, 2 experiments were conducted to determine if dietary enzymes increased the digestibility of nutrients bound by non-starch polysaccharides (NSP) and phytate in millrun and consequently improved performance. Xylanase improved ($P < 0.05$) total tract energy digestibility, DE content and G:F. Phytase reduced ($P < 0.05$) ADFI, and xylanase tended to reduce ($P = 0.07$) ADFI. In Chapter 2, effects of xylanase on nutrient digestibility were studied in a wheat control diet and 5 diets containing 30% by-product (millrun, middlings, shorts, screenings, and bran). Xylanase improved ($P < 0.05$) total tract energy digestibility of the millrun, shorts, screenings, and bran diets. Xylanase did not affect hindgut fermentation but reduced ($P < 0.05$) hindgut fermentable DE. In Chapter 3, effects of supplementing xylanase and (or) phytase on nutrient digestibility, digesta passage rate and mean digesta retention time (MRT) of a wheat-based diet containing 20% millrun were investigated. The enzymes interacted to increase ($P < 0.05$) total tract nutrient digestibility and DE content of the negative control diet, but did not affect passage rate and MRT. In Chapter 4, effects of xylanase and phytase supplementation on site of nutrient digestibility in weaned pigs, pH content in the gastrointestinal tract and on growth performance were studied in diets with reduced nutrient specifications (negative control: NC). Xylanase improved ($P < 0.01$) energy digestibility of the NC in the mid jejunum and over the total tract by 63.0 and 4.6%, respectively. Diet tended to reduce ($P = 0.074$) the pH content of the upper small intestine, and phytase raised ($P < 0.01$)

the pH content of the upper mid small intestine. Both enzymes improved total tract DE content and performance of weaned pigs. Phytase inclusion led to a more rapid return to alkaline conditions in the upper part of the small intestine. In conclusion, the nutritional value of millrun can be improved with exogenous enzymes thereby improving its status as an opportunity feedstuff in swine diets.

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DEDICATION

This thesis is dedicated to my parents Thomas and Beatrice Nortey, and also to my brother and sisters, Henry, Doris, and Bernadette.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA amino acid

ADF acid detergent fibre

ADFI average daily feed intake

ADG average daily gain

AID apparent ileal digestibility

ANF anti-nutritional factor

BW body weight

CF curde fibre

CP crude protein

DE digestible energy

DM dry matter

EE ether extract

GE gross energy

G:F feed efficiency

ME metabolizable energy

MRT mean retention time

NDF neutral detergent fibre

NSP non-starch polysaccharide

SEM standard error of the mean

U unit

VFI voluntary feed intake

1. LITERATURE REVIEW

1.1 Introduction

Feed costs account for approximately 50 to 70% of the variable production cost in intensive swine production. The major components of most swine rations are cereals, which are an important energy source. In Alberta and Saskatchewan, the most commonly used cereal in swine diets is wheat (Figure 1-1), while less commonly used cereals include barley, hull-less barley, corn, and oat. A possible approach for reducing the relatively high feed cost component in commercial swine production is effective utilization of wheat by-products in swine diets.

Wheat by-products from dry milling result from processing wheat into the final product flour for human consumption. These by-products are not widely used for human foods, and animal agriculture serves a useful function by using by-products as feedstuffs, thereby converting low-value plant-based by-products into high-value animal products. Generally, wheat by-products have a higher content of fibre, crude protein, and minerals than the parent grain, because much of the starch fraction has been removed during the milling process (Holden and Zimmerman, 1991). Plant carbohydrates such as starch and fibre are quantitatively the most common energy source for monogastric species (Bach Knudsen and Canibe, 2000). However, monogastric species do not digest high-fibre feedstuffs well; thus, the digestible energy (DE) content of most grain by-products is low for swine (Holden and Zimmerman,

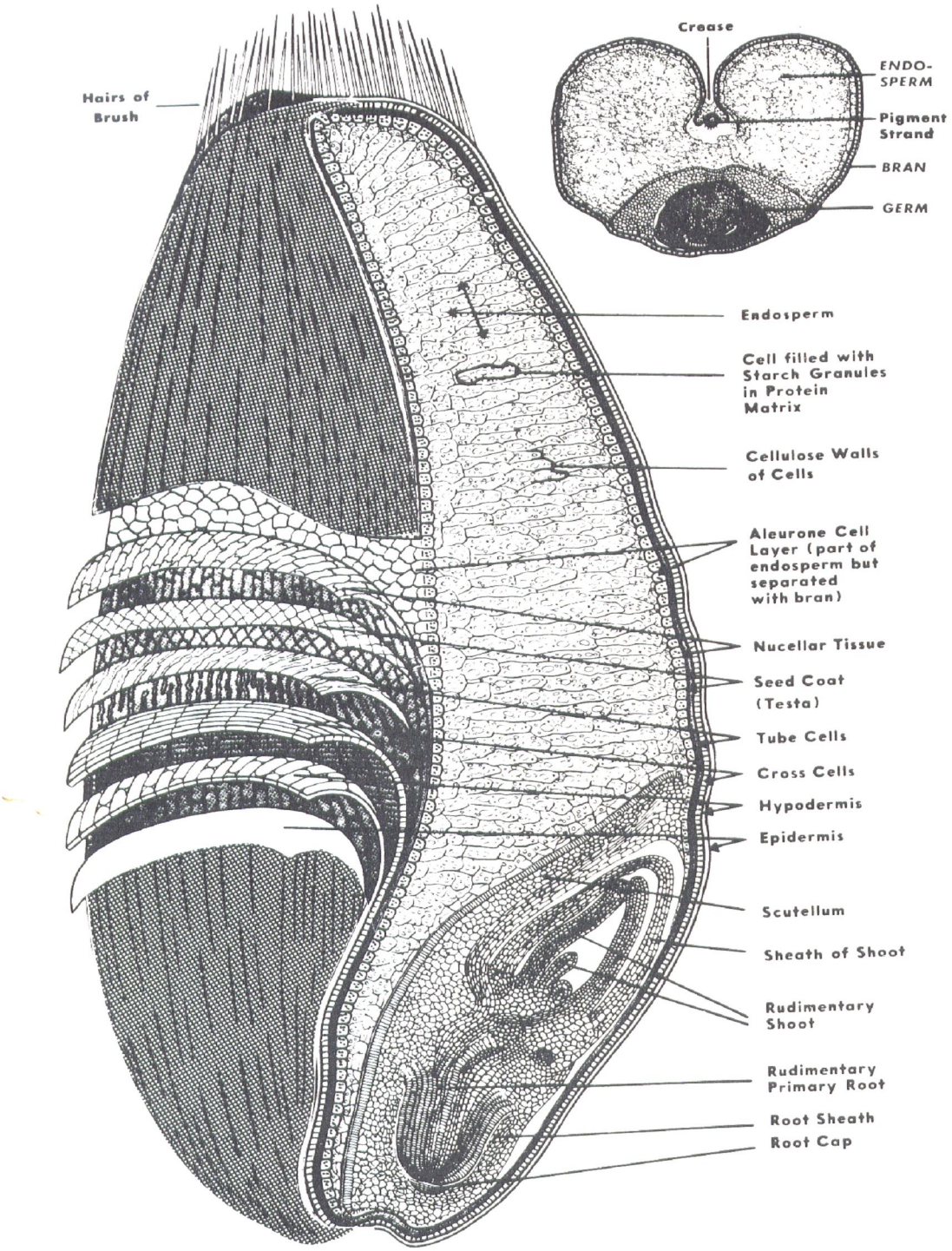

Figure 1-1 Longitudinal and cross sections of a wheat kernel

1991). Although some of the grain by-products are concentrated sources of protein, the quality of this protein may be poor, due to deficiencies in some of the essential amino acids such as Lys, Thr, and Trp (Holden and Zimmerman, 1991). Additionally, amino acid digestibility in wheat by-products may be lower than in the parent wheat (Lin et al., 1987; Sauer et al., 1977). The reason for the reduced digestibility is that most of the nutrients contained in high fibre diets are encapsulated within a fibre matrix. Pigs do not produce the enzymes required to breakdown this matrix (Barrera et al., 2000). As a result, these nutrients are unavailable in the upper gastro-intestinal tract, the site for nutrient absorption for efficient support of protein deposition (Noblet, 1994).

Phosphorous is the mineral with the highest economical value in wheat by-products; however, P digestibility is poor in pigs. In plants-based feedstuffs, a large proportion of P is present as phytate P (Adeola et al., 2004). Pigs have a limited ability to digest phytate-P because endogenous phytase necessary for hydrolysis of phytate is lacking (Golovan et al., 2001). Therefore the availability of plant P to pigs is low.

Limited research has been published concerning the value for pigs of enzyme supplementation of diets on wheat by-products, especially millrun, which is a feedstuff that includes the various wheat by-products from dry milling. Furthermore, results of experiments using wheat by-products and enzymes have not been consistent. With the increasing need to explore alternative and cost-effective feedstuffs, research into the use of wheat by-products as a feedstuff in swine diets is of great interest.

1.2 Wheat By-Products in Diets for Growing Pigs: A Review

1.2.1 Definitions of wheat by-products

Processing wheat into flour for human consumption also produces wheat by-products. The dry milling process that converts whole wheat grain into flour and accompanying by-products includes several steps (Candlish, 1997). The wheat is initially cleaned to remove foreign material, and badly damaged shrunken kernels. The wheat is then tempered, to the optimum moisture content for milling by the addition of water. Milling breaks open the seed, scrapes off as much of the bran as possible and grinds the endosperm into flour (Figure 1-2-1). The wheat is ground on break rolls and reduction rolls. Subsequently, flour is separated using purifiers and plan sifters. Four distinct systems exist in the flour milling process: break, grading, purification, and reduction systems. The ground material leaving each break roll passes to the sieving system, which separates the mixture of particles according to size using sifting machines. The coarse co-product from the break system is called bran and the finer bran-like material from the purification and reduction system is known as shorts. The definition of wheat by-products from dry milling depends mainly on their chemical characteristics and geographical region.

***Wheat bran*.** According to the Association of American Feed Control Officials (AAFCO, 1988), wheat bran is the coarse outer covering of the wheat kernel as separated from cleaned and scoured wheat in the usual process of commercial milling. Wheat bran consists of the outer covering of the wheat kernel together with small amounts of flour and finely ground weed seeds (Holden and Zimmerman, 1991). Wheat bran contains a minimum of 13.5 to 15.5% crude protein (CP), a minimum of 2.5%

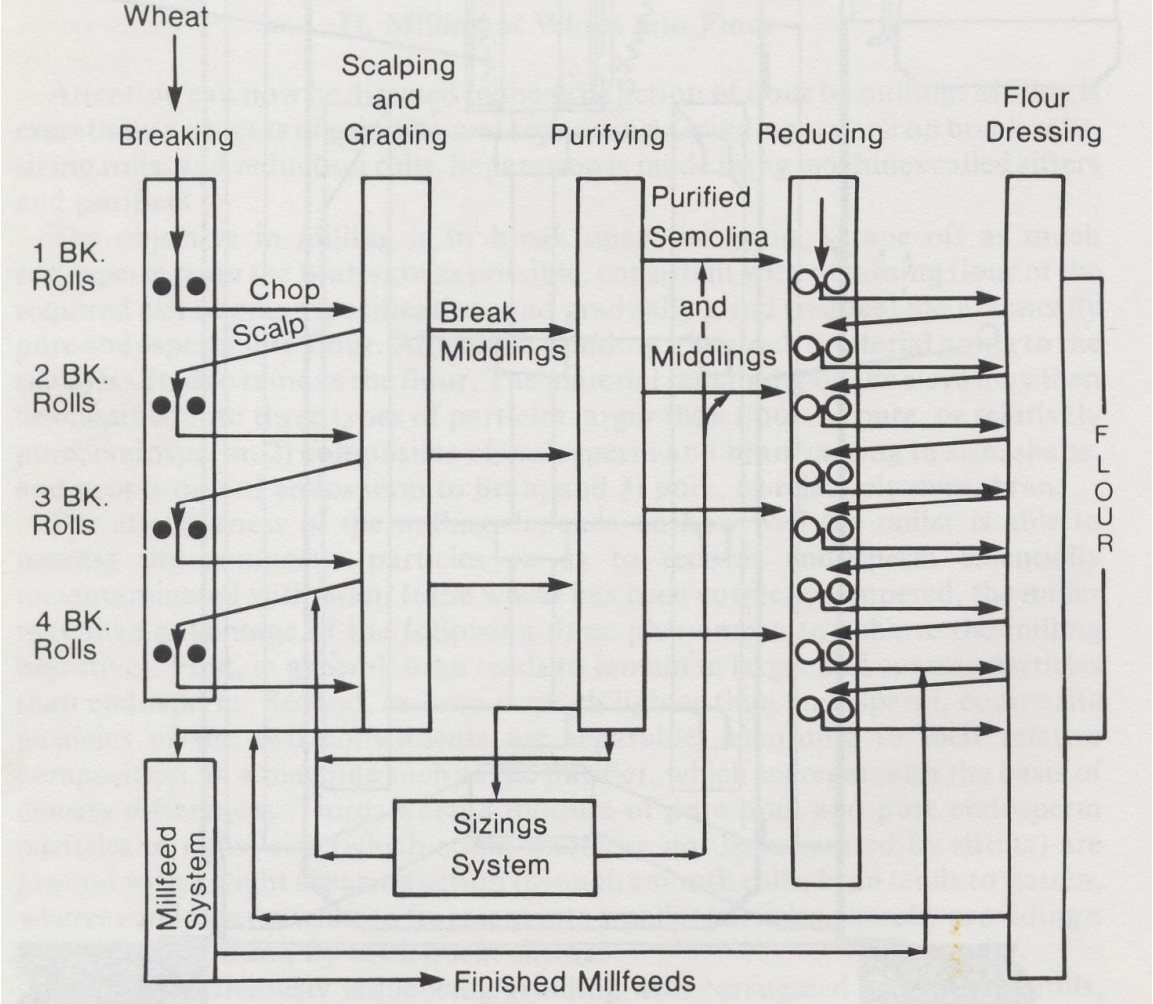

Figure 1-2 Schematic diagram of a simple mill flow

ether extract (EE), and a minimum of 12% crude fibre (CF), and is a feedstuff for swine diets. Wheat bran is a product with a low density that has traditionally been used in diets for sows as a laxative. Wheat bran is also defined (Ewing, 1997) as a by-product of flour manufacture, obtained from screened grains of wheat or dehusked spelt. Wheat bran consists principally of fragments of the outer skins and of particles of grain from which the greater part of the endosperm has been removed. Wheat bran contains the pericarp and testa or coarse bran.

Wheat shorts. Shorts consist of fine particles of wheat bran, wheat germ, wheat flour, and the offal from the "tail of the mill". Wheat shorts must be obtained in the usual process of commercial milling and must contain not more than 7% CF (AAFCO 1988). Wheat shorts is the layer of the wheat kernel just inside the outer bran layer covering the endosperm (Huang et al., 1999) and shorts usually contain 5 to 10% CF and 15 to 20% CP. Wheat shorts are used as an ingredient for swine.

Wheat middlings. Middlings consist of fine particles of wheat bran, wheat shorts, wheat germ, wheat flour, and some of the offal from the "tail of the mill" (AAFCO, 1988). Wheat middlings must be obtained in the usual process of commercial milling and must contain not more than 9.5% CF. Wheat middlings are a by-product of the wheat milling industry, consisting mostly of fine particles of bran and germ (O'Hearn and Easter, 1983). Middlings are classified to contain at least 15.5% CP, 88% dry matter (DM), and not more than 7.5% CF.

Wheat millrun. Millrun consists of coarse wheat bran, fine particles of wheat bran, wheat shorts, wheat flour, and the offal from the "tail of the mill". Millrun must be obtained in the usual process of commercial milling and contain not more than 9.5% CF. During the milling process, individual by-products have largely lost their identity and therefore many flourmills combine all by-product streams with the screenings (Dale, 1996).

Wheat red dog. Red dog consists of the offal from the "tail of the mill" together with some fine particles of wheat bran, wheat germ, and wheat flour (AAFCO, 1988). Red dog must be obtained in the usual process of commercial milling and must contain not more than 4% CF.

Wheat germ meal. Germ meal consists chiefly of wheat germ together with some bran and middlings or shorts. Germ meal must contain not less than 25% CP and 7% EE.

Defatted wheat germ meal. Defatted germ meal is obtained after the removal of part of the oil or fat from wheat germ meal and must contain not less than 30% CP (AAFCO, 1988).

Wheat feed. Wheat feed is a by-product of flour manufacture from screened grains of wheat or de-husked spelt. Wheat feed consists principally of fragments of the outer skins and of particles of grain that have less of the endosperm removed than wheat bran (Ewing, 1997). Wheat feed is the "family" name given to all offal from screening and de-husking of wheat from the flour milling industry including wheat bran, endosperm, and other screenings. For every metric ton of wheat processed, approximately 20 kg of wheat feed is produced. Wheat feed is highly digestible, but quality varies among production plants. Wheat feed is ideal for swine feed and is a good source of P, but is low in Ca, Na, and vitamins.

Wheat distiller's dark grains. Distiller's dark grains is a by-product of alcohol distilling (whisky, gin, and vodka) obtained by drying solid residues of fermented grain that have pot ale syrup or evaporated spent wash added (Ewing, 1997). To obtain wheat distiller's dark grains, wheat is soaked to release the starch reserves for fermentation. Often, some barley malt is added to start the process, providing enzymes to convert starch into sugar. The grain that remains after the liquor is removed is often called wet draff. The draff is pressed and dried with the leftover yeast syrups to produce dark grains. Distiller's dark grain is high in energy and protein; the protein is partly

degraded. Distiller's dark grain contains digestible fibre at moderate levels, making it ideal for ruminant species but less suitable for non-ruminant species.

Wheat distiller's dried grain with solubles. Distiller's dried grain with solubles (DDGS) is a by product of the ethanol and alcohol beverage-based industries that rely on grain as raw materials (Newland and Mahan, 1990). Work done by Widyaratne and Zijlstra, (2007) indicates that the crude protein, crude fat and crude fibre contents of DDGS is 44.5, 2.9, and 7.6% respectively. As part of a trial to study the energy and nutrient digestibility of DDGS fed to grower pigs, Nyachoti et al. (2005) determined that the total tract DE, N, P and Ca contents of winter wheat DDGS were 13.4 MJ/kg, 49.4, 4.9, and 0.96 g/kg respectively.

1.2.2 Nutrient content and digestibility of wheat by-products in swine diets

1.2.2.1 Energy content and digestibility

Wheat by-products generally have a higher fibre content compared to the parent grain, are therefore not as well digested by swine, and have a lower energy density (Holden and Zimmerman, 1991). For example, wheat (hard red winter) contains 13.5% neutral detergent fibre (NDF), 4.0% acid detergent fibre (ADF) and 3365 kcal/kg digestible energy (DE), whereas wheat middlings and wheat shorts contain 35.6%, 23.4%, and 3075 kcal/kg, and 10.7%, 8.6%, and 2985 kcal/kg of NDF, ADF, and DE respectively (NRC, 1998).

Plant carbohydrates are quantitatively the most important energy source for non-ruminant species (Bach Knudsen and Canibe, 2000). In cereals, the carbohydrate fraction includes two broad classes, i.e., starch and the polysaccharides of the cell wall.

The latter are being referred to as non-starch polysaccharides (NSP) or fibre. Cell wall materials from various cellular tissues of cereals may potentially influence the digestion and absorption processes in various ways.

Most of the starch in cereals has an open structure enabling easy access to hydrolysis by starch-degrading enzymes (α-amylases) in the porcine gastro-intestinal tract. However, porcine endogenous enzymes are not able to degrade the glycosidic linkages in NSP. The bulk of dietary NSP is recovered in the ileum and the breakdown of NSP and resistant starch by the colonic microflora depends on the type of polymer and the degree of lignification.

The breakdown of plant carbohydrates in the digestive tract of pigs has been studied extensively (Bach Knudsen and Canibe, 2000). For example, 3 diets including 1 low-fibre diet and 2 high-fibre diets with either wheat bran or oat bran were formulated to provide 30% energy from fat, 18% energy from protein, and 50% energy from carbohydrates, and with variable ratios between starch and NSP. Diets were fed to 8 ileal-cannulated hypercholesterolemic pigs in a cross-over design. Oat bran that contained a high level (7.6%) of soluble digestible fibre in the form of β-glucan and wheat bran that contained a high level (44.8 %) of insoluble digestible fibre in the form of arabinoxylans, cellulose, and lignin was used to raise the digestible fibre level. Carbohydrates were the predominant constituents of ileal digesta, with most of the carbohydrates present as NSP. Starch in all diets was almost completely digested in the ileum, with digestibility values above 99%. The ingested amount of NSP was quantitatively recovered in ileal digesta from the wheat-based diets, while 27% of NSP, primarily mixed linked (1→3) (1→)-β-D-glucans, was digested for the oat bran diet.

The concentration and flow of lactic acid were highest in the ileum after feeding the oat bran diet, indicating that β-glucans stimulated lactic acid formation. Any soluble NSP that reached the large intestine were almost completely degraded, while the digestibility of insoluble NSP was lower for the low fibre and wheat bran diet than for the oat bran diet. Oat bran degraded at a fast rate and therefore reduced the expected increase in wet and dry feces mass that was observed with wheat bran. Cereal starch was therefore practically completely degraded in the small intestine. Fibre source influenced the site and extent of NSP degradation, lactic acid formation, and bulking properties. The insoluble NSP that form the bulk of NSP in wheat cannot be broken down by the endogenous enzymes in the pig, and are mostly degraded in the large intestine (Bach Knudsen and Canibe, 2000). On the other hand a small amount of soluble NSP like β-glucan is hydrolyzed in the small intestine.

The origin and composition of cell walls can also cause large variations in their utilization. For example, the insoluble, lignified NSP contained in the secondary cell walls are relatively resistant to microbial degradation in the gastro-intestinal tract of swine, while the non-lignified NSP contained in the cell walls and middle lamellae are more susceptible to microbial attack (Graham et al., 1986). The digestion of four fibre sources, representing four sources of plant cell walls (wheat bran, sugar beet pulp, soybean hulls, and wheat straw) was studied in grower pigs by partially replacing starch (Chabeuti et al., 1991). Regardless of the fibre source added to the diets, the apparent digestibility of N and GE was reduced relative to the control diet. The apparent digestibility of NDF differed and reflected total NSP content of the fibre source. The total NSP contents were in the order soybean hulls > sugar beet pulp > wheat bran >

wheat straw. Increasing the amounts of wheat bran in diets reduced digestibility of total NSP and ADF. In diets with similar amounts of NSP, plant cell walls from sugar beet pulp and soybean hulls were highly digestible (69 and 75% for total NSP, respectively), intermediate for wheat bran (51%), while NSP from wheat straw were the least digestible (30%). The results indicate that digestibility of cell wall components from different sources varies in pigs.

Feeding high fibre diets to growing-finishing pigs may reduce energy intake, due to energy density dilution, and subsequently decreased body fatness. The effects of dietary fibre from wheat bran on digestibility of nutrients, and performance were studied in European Large White and Chinese Mei Shan pigs (Fevrier et al., 1992). Inclusion of 20 or 52% wheat bran into diets reaching 5.1 and 7.9% ADF, respectively, depressed the apparent digestibility of energy for both breeds. For an increase of 1% in ADF, the digestibility of energy decreased by 4.8 or 2.8% units respectively. The apparent digestibility of cellulose or hemicellulose was similar; however, both decreased linearly with increasing dietary ADF content. Mei Shan pigs utilized fibrous components better, only in the case of 52% wheat bran, and fibre content did not affect fatness of the pigs. In summary inclusion of high levels of fibre in diets for growing pigs may affect the digestion of other nutrients. In addition, different breeds of pigs have different abilities to digest fibre.

The relationship between crude fibre content and apparent ileal and total-tract digestibility of amino acids (AA), gross energy (GE) and starch was investigated in corn, sorghum, wheat, barley, oat groats, and wheat middlings in growing pigs (Lin et al., 1987). These feedstuffs represent a wide range in crude fibre content and

carbohydrate profiles. Total-tract digestibility of GE was similar for corn, sorghum, and oat groats (91.4 to 91.9%), slightly lower for wheat (88.1%), followed by barley (79.5%), while wheat middlings had the worst energy digestibility (66.9%). The amount of dietary GE digested in the large intestine was lowest, and similar for corn, sorghum, wheat and oat groats (averaging 7.0%), medium for barley (11.0%), while the greatest amount of digestion occurred for wheat middlings (17.0%). The amount of energy digested in the large intestine was inversely related to ileal energy digestibility. The higher degradation rates for barley and wheat middlings reflect a high content of fibre, which was degraded by the microflora of the large intestine. Therefore high fibre diets fed to pigs shift the site of digestion from the upper part of the GIT (endogenous enzymatic hydrolysis) to the large intestine where microbial fermentation is predominant.

For efficient use of wheat by-products, knowledge of its precise nutrient content and availability is needed. Using wheat shorts included in diets based on corn and soybean meal, the DE content of wheat shorts was measured to be between 2.91 and 2.94 Mcal/kg DM, using regression analysis (Patience et al., 1977a). A suggested, acceptable level of inclusion of wheat shorts in diets for grower pigs is less than 20% (Patience et al., 1977a).

In summary, cell wall materials influence digestion and absorption of nutrients. Starch is almost completely degraded in the small intestine regardless of NSP content of diets, while NSP degradation, lactic acid formation and bulking properties are influenced by fibre source. Increasing the fibre level in a swine diet, regardless of the source of fibre may reduce the apparent ileal digestibility of energy.

1.2.2.2 Protein and lysine content and digestibility

Wheat by-products have a higher content of CP (Slominski et al., 2004) and AA than the parent wheat. For example, wheat (hard red winter) contains 13.5% CP and wheat shorts and wheat middlings contain 16 and 15.9% CP, respectively (NRC 1998). However, digestibility of CP and AA may be lower in the by-products than in the wheat due to higher fibre concentrations.

The inclusion of wheat by-products in diets for growing pigs will reduce apparent digestibility of N. The addition of four different fibre sources (wheat bran, sugar beet pulp, soybean hulls, and wheat straw) reduced the apparent digestibility of N compared to a control diet based on wheat and starch as the only sources of carbohydrate (Chabeauti et al., 1991). The total-tract apparent digestibility of N was 92% for the basal diet and 87 or 79% for wheat bran, 83% for sugar beet pulp, 81% for soybean hulls, and 81% for wheat straw. Clearly, dietary fibre has an inverse relationship with N digestibility.

A common wheat by-product used in diets for grower-finisher pigs is wheat middlings. Wheat middlings varies in nutritional composition, partly resulting from differences in the amount of starch-containing endosperm remaining from the parent grain during the milling process. Thus, wheat middlings containing more of endosperm are superior in nutritional value for grower-finisher pigs than middlings containing less endosperm (Cromwell et al., 2000). The variability among sources of wheat middlings and nutrient analyses were studied among 20 laboratories (Cromwell et al., 2000). The bulk density of the middlings ranged from 289 to 365 g/L. The middlings averaged

16.2% CP, 0.66% Lys, 0.19% Trp, 0.54% Thr, 0.25% Met, 0.34% Cys, 0.50% Ile, and 0.73% Val. Thus sources of wheat by products including middlings vary in chemical composition depending on their bulk density that reflects the proportion of bran and flour in the middlings.

As a processed grain by-product, wheat middlings tend to be variable in nutrient composition partly because millers occasionally increase the portion of the by-product that is selling at the highest price and (or) volume. Thus, variations in nutrient composition of middlings, and subsequent growth performance of pigs fed diets containing middlings are not unusual (Erickson et al., 1985). In a balance trial conducted to evaluate wheat middlings processed from Michigan soft white winter wheat in pelleted swine diets, the addition of middlings did not reduce apparent CP digestibility (Erickson et al., 1985). Inclusion of 20% middlings in the diet maximized the indicators apparent biological value and net protein value, whereas 40 and 60% middlings depressed these indicators.

Part of the variation in AA digestibility of wheat by-products may be related to differences in NDF content (Huang et al., 2001). The relationship exists because significant amounts of N and AA are associated with the NDF fraction of feedstuffs (Schulze et al., 1994; Lenis et al., 1996). Protein and AA associated with the NDF fractions likely have a low digestibility, because the digestive enzymes have limited access to the cell wall contents. Simulated samples of wheat shorts consisting of different portions of wheat bran, wheat shorts, and wheat flour were created to study variability in apparent ileal AA digestibility (Huang et al., 1999). The proportions of wheat screening, wheat bran, and wheat flour and the NDF content (DM basis) of the

wheat fractions were as follows: A, 70% wheat screening and 30% wheat bran, 42.3% NDF; B, 85% wheat screening and 15% wheat bran, 41.8% NDF; C, 100% wheat screening, 41.3% NDF; D, 85% wheat screening and 15% wheat flour, 35.2% NDF; and E, 70% wheat screening and 30% wheat flour, 29.5% NDF. The apparent ileal digestibility of AA in the wheat fractions was calculated using the difference method (Fan and Sauer, 1995). Apparent ileal digestibility of CP for wheat shorts in wheat fraction A, B, C, D, and E were 59.2, 61.4, 62.9, 68.6, and 67.6%, respectively. The digestibility was lowest in the wheat fractions containing wheat bran and highest in fractions without wheat bran. The average digestibility of indispensable AA was 63.5% for wheat fraction A, which contained 30% wheat bran, and 71.9% for wheat fraction C, which did not contain wheat bran. The Lys digestibility did not differ among the wheat fractions, and ranged from 54.7 to 64.1%. Of the indispensable AA, with the exception of Arg, Lys, and Met, the apparent ileal digestibility values of AA were negatively correlated with the NDF content in the wheat fractions.

The feeding value of wheat by-products in general and wheat shorts specifically as a major ingredient in the animal feed industry was investigated previously (Patience et al., 1977a). Supplementation of wheat shorts with varied levels of corn and 2 levels of soybean meal indicated a major limitation of protein quality with respect to the requirements of grower-finisher pigs. The addition of soybean meal improved protein digestibility indicating a lower digestibility of protein in wheat shorts. The N digestibility coefficient of wheat shorts was 71%. For the diets with 0, 15, 30, and 45% added corn, the N digestibility coefficients were 71.2, 71.8, 72.4, and 69.2%, respectively, for the 0% added soybean meal, and 76.5, 78.1, 78.0, and 76.1%,

respectively, for the 10% added soybean meal. Shorts were first limiting in protein quality for the market hog.

The limiting AA in wheat shorts fed to swine was also investigated (Patience et al., 1977b). Supplementation of Lys and/or Thr for the pig showed equal limitation of these AA with respect to growth performance and fasting plasma urea nitrogen (PUN). However post-prandial PUN, N balance and urine urea measurements indicated that Lys was first limiting and Thr second limiting for swine. Also, the apparent digestibility of protein in shorts was only 75% in swine. Generally, increasing the proportion of wheat by-products (and consequently the fibre content) in a diet for swine results in a reduction in the apparent digestibility of protein and AA.

1.2.2.3 Phosphorous content and digestibility

In most plant materials, a large proportion of P is in the form of phytate (Ravindran et al., 1994). Phytate is a complex salt of calcium and magnesium with myoinositol (1,2,3,4,5,6 hexakis dihydrogen phosphate) and is regarded as the chief storage form of P and inositol in seeds and vegetative storage tissues. In mature seeds, phytate-P is present as a complex salt of calcium, magnesium and potassium, and/or with proteins (Ravindran et al., 1995). In general the proportion of phytate P in seeds of cereals, grain legumes and oil-bearing plants varies from 60 to 80% of the total phosphorous in these materials. Phytate binds essential dietary minerals, making them unavailable or only partially available for uptake (Ravindran et al., 1994, 1995).

Some bran sources can contain over 5% phytic acid (Garcia-Estepa et al., 1999). Consequently, increasing the inclusion rate of wheat by-products in diets for swine will

increase the consumption of phytic acid. The pig has a limited ability to utilize phytic P (Jendza et al., 2005); thus, supplementing cereal diets high in phytic acid with the enzyme phytase can improve the overall availability of P.

Wheat by-products have a higher P and phytic acid content than the parent grain. For example, wheat (hard red winter) contains 0.37% P and wheat shorts and wheat middlings contain 0.84 and 0.93% P, respectively (NRC, 1998). For precise feed formulation for swine, accurate chemical analysis of feed ingredients is important. However, wide variation exists in P content among wheat by-product samples and also among laboratories. For example, the P content among samples of wheat middlings ranged from 0.70 to 1.19%, while the coefficient of variation among laboratories for an individual sample was 9.15 (Cromwell, 2000).

The P digestibility of plant-based ingredients may be low. Phytic acid (myoinositol hexa-phosphoric acid, IP6) is the major P storage compound of most seed and cereal grains and may account for more than 70% of the total P (Garcia-Estapa et al., 1999). Apart from binding P, phytic acid has a strong ability to chelate multivalent metal ions, especially Zn, Ca, and Fe. The binding can result in insoluble salts with poor availability of included minerals.

Among the wheat by-products, phytic acid is contained in the wheat bran, and increased dietary inclusion of bran can result in lowered P availability, because the bran may contain more than 5% phytic acid. The phytic acid content for wheat bran ranged from 25.3 to 58.4 mg/g, depending on wheat variety (Garcia-Estapa et al., 1999). In contrast, the phytic acid content averaged 21.5 mg/g for oat bran and 57.7 mg/g for rice bran.

Similarly, the distribution of phytic acid in milled fractions of hard red winter wheat (var. Scout) was measured using various analytical methods (Lehrfield and Wu, 1991). The whole wheat contained 1.03% phytic acid. In contrast, the phytic acid content of the milled fractions varied from less than 0.08% in the reduction and break flours to 1.05% in the shorts and 4.06% in the bran. The largest bran particles contained 4.99% phytic acid, whereas the small particles contained 1.07%. Overall, the phytic acid is clearly unevenly distributed within the wheat kernel.

The availability of P in wheat by-products varies widely. For example, P availability was 41% for wheat middlings and 29% for wheat bran (NRC, 1998). Wheat middlings containing 0.9% P and processed from Michigan soft white winter wheat were used to partially replace corn, dehulled soybean meal, dicalcium phosphate, and Ca carbonate and to function as a pellet binder in grower and finisher swine diets (Erickson et al., 1985). Wheat middlings replaced corn on an equal-weight basis at 0, 20, 40, and 60% of the diet for a total P content of 0.64, 0.73, 0.80, and 0.88 versus 0.57, 0.64, 0.73, and 0.81% respectively for the grower and finisher pig diets. Although carcass length was similar among dietary treatments, metatarsal ash decreased linearly from 59.9 to 58.2% with increasing inclusion rates of middlings, indicating that availability of P was lower in wheat middlings than in corn.

Supplemental microbial phytase in diets for pigs is an effective way of improving bioavailability of dietary phytate P and other nutrients (Han et al., 1998). Wheat by-products contain relatively high intrinsic phytase activity and the collective efficacy of cereal phytase from wheat middlings, microbial phytase, and citric acid in improving phytate-P bioavailability was investigated using practical corn-soybean diets in 2 pig

experiments (Han et al., 1998). In study 1, 40 gilts were fed 5 diets based on corn and soybean meal for 8 wk. Diets 1, 2, and 3 were low-P and contained 0, 0.1, or 0.2% inorganic P as calcium phosphate, respectively. Diet 4 contained 15% wheat middlings (461 cereal phytase U/kg). Diet 5 contained microbial phytase (1,200 U/kg). Pigs fed the low P diet without inorganic P (Diet 1) had lower indices of P status including a lower ADG, ADFI, plasma P concentration, bone strength, and mobility score than pigs fed the other 4 diets. For pigs fed the wheat middling diet, these indices were not different from pigs fed the diet with 0.1% P or microbial phytase. In study 2, 40 barrows and gilts were fed 4 diets for 6 wk. Diet 1 contained 0.2% P, diet 2 contained 300 U/kg microbial phytase, diet 3 contained 300 U/kg microbial phytase plus 1.5% citric acid, and diet 4 contained 300 U/kg microbial phytase, 1.5% citric acid, and 10% wheat middlings. Pigs fed diet 4 had a higher feed efficiency than pigs fed diet 1, during wk 1 to 3, suggesting that calcium phosphate might be replaced with 10 to 15% wheat middlings, 300 U microbial phytase/kg, and 1.5% citric acid in corn-soy diets for grower pigs (Han et al., 1998).

The intrinsic phytase activity of different plants varies considerably (Helander et al., 1994). Wheat is a feedstuff with a high phytase activity, ranging from 300 to 2000 IU/kg. Most of this wheat phytase appears in the surface layers. The effect of intrinsic phytase in wheat bran on phytin-P availability was studied in a 2 x 3 factorial arrangement in pigs fed barley-based diets with and without wheat bran and 3 P levels [4.3, 3.0 and 1.6 g digestible P per feed unit (FU = 0.7 kg starch equivalent)]. Wheat bran inclusion in the diet did not affect dietary P utilization. However, wheat bran

inclusion improved absorption and retention of P in the low P diet. Intrinsic phytase included in wheat bran may thus improve P availability.

The P in plant feedstuffs is found in 2 separate groups: organically bound P present as phytic acid and P present as non-phytate P (Vivero et al., 2000). Phytases occur naturally in seeds but their activity is poorly understood. Samples from feedstuffs used in the animal feed industry were collected and the total P and phytate P contents and activities for phytase and acid phosphatase were determined. For wheat bran, total P was 1.16%, phytate P was 0.88% and activities of intrinsic phytase and acid phosphatase were 4624 and 14,106 U/g, respectively (Vivero et al., 2000).

In summary, wheat by-products contain high levels of P; however the P is mostly bound as phytic acid and is therefore not readily available to pigs. Increasing levels of wheat by-products in diets for pigs may increase P availability due to the presence of intrinsic phytase from the wheat by-product.

1.2.3 Impact of diets containing wheat by-products on animal growth, performance and carcass characteristics

1.2.3.1 Voluntary feed intake

Voluntary feed intake is an important consideration in developing appropriate diets that meet the requirements of pigs in achieving targeted growth rates and improving overall efficiency of swine operations. The inclusion of wheat middlings in diets for pigs increased feed consumption (Erickson et al., 1985). Replacing corn with 0, 10, 20, and 30% wheat middlings (wt/wt) increased feed consumption (1.82, 1.85, 1.85, and 2.00 kg/d, respectively) for pigs.

Two growth trials were conducted with grower pigs to determine the influence of level of wheat shorts, Lys addition and pelleting of diets on performance (Young, 1980). Four levels of wheat shorts (0, 32.2, 64.4, and 96.6% of the diet), 2 levels of Lys addition (0 and 0.11%) and 2 physical forms of the diet (meal and pellets) were used. Although ADG and feed efficiency (G:F) declined with increasing dietary levels of wheat shorts above 64.4%, ADFI did not differ among the treatments. The ability of a pig to consume bulky diets is limited by the physical capacity of the pig to ingest the particular feed (Nyachoti et al., 2004). Therefore with reduced energy and nutrient density, and a simultaneous inability to consume more of the feed, performance parameters like ADG and G:F may decline with increasing amounts of bulky ingredients in the diet.

In summary, the effects of including wheat by-products in swine diets are contradictory. The literature suggests that the level of wheat by-products that is included in the diet for pigs does not affect ADFI and this may be due to limitations imposed by the physical inability of the pig to consume more feed to compensate for a bulkier feed.

1.2.3.2 Weight gain

An increase in ADG or growth rate improves efficiency of commercial pork production (Nyachoti et al., 2004). In a balance trial conducted to evaluate wheat middlings processed from Michigan soft white winter wheat in pelleted swine diets, the addition of middlings did not reduce apparent CP digestibility. Inclusion of 20% middlings in the diet maximized the indicators apparent biological value and net protein value, whereas 40 and 60% middlings depressed these indicators. Increasing the dietary inclusion (0,

10, 20, and 30%) of pelleted wheat middlings processed from soft white winter wheat (Erickson et al., 1985) resulted in an increase in ADFI during the finisher phase. However this increase in intake was not enough to cause an increase ADG of pigs (0.65, 0.66, 0.67, and 0.66 kg/d respectively). In an experiment to study whether wheat bran phytase could improve the availability of intrinsic phytase in a commercial barley-soybean meal diet with either a high (4.33 g) or low (2.99 g) digestible P/kg content, Helander and Partanen (1994) found that increasing the dietary inclusion of wheat bran in grower pig diets from 0 to 10% did not affect the ADG over the 30 to 100 kg liveweight range.

Studies have been conducted to investigate the effects of wheat shorts on the performance of growing pigs. The effects of replacing a corn-soybean meal diet with graded levels of wheat shorts (0 to 96.9 %) were studied in growing pigs (Patience et al., 1977a). Supplementation of the diet with up to 20% wheat shorts did not affect the ADG; however with a higher inclusion rate, ADG tended to reduce. Furthermore, the effects of three levels of corn (0, 30, and 45%) and two levels of soybean meal (0 and 10%) in a diet based on wheat shorts for growing pigs was studied. The addition of soybean meal improved protein digestibility, indicating a lower digestibility of the protein in the wheat shorts, and increased ADG.

In another trial, Patience et al. (1997b) investigated limiting AA in wheat shorts fed to swine. Thr and Lys were chosen for consideration as limiting AA in swine diets based on earlier trials using male weanling rats. Addition of either Lys or Thr produced insignificant depressions in growth parameters. However adding both AA to the diet

produced an increased rate of gain. This indicates that both Lys and Thr are equally limiting in a diet based on wheat shorts.

From the trials above it may concluded that wheat shorts may be included up to 30% in diets for finishing pigs. To maintain growth performance, the amino acids Thr and Lys may be supplemented in the diet, since both are limiting in diets based on wheat shorts.

In pork production, feed costs might be reduced by replacing regular feed ingredients such as corn with wheat by-products in the diet. The inclusion of 5, 15, and 30% wheat middlings in diets fed to pigs from 28 to 65 kg reduced ADFI and G:F but not ADG (Shaw et al., 2002). Furthermore, ADG was not affected by 5 to 15% wheat middlings in the nursery phase or by 30% of wheat middlings from 65 kg to slaughter. Increasing dietary inclusion of wheat shorts above 64.4% reduced ADG and G:F (Young, 1980). Thus ADG of pigs is generally not affected by dietary levels of wheat by-products provided that the inclusion levels are low (about 30%). This is generally due to the lower digestibility of nutrients including amino acids in wheat by-products. In summary pigs fed diets with 30% or more wheat by-products in the diets may have lower ADG. This is because high levels of fibre can prevent the digestion of nutrients some of which are enclosed within their matrix.

1.2.4 Effects of supplemental enzymes to wheat by-product diets

1.2.4.1 Fibre-degrading enzymes

Feed ingredients of plant origin contain a number of components such as anti-nutritional factors (ANF) that cannot be digested by monogastric species because of the lack of, or insufficiency of, endogenous enzyme secretions. Examples of ANF include pentosans in wheat and β-glucans in barley (Ravindran et al., 1999).

The efficacy of fibre degrading enzymes in high fibre based diets for monogastric livestock is based broadly on two principles: viscosity reduction of digesta and demasking (Brufau, 2006). Viscosity relates to more soluble NSP like β-glucan and is more of a concern in poultry. The NSP cannot be hydrolyzed by the endogenous enzymes in birds. The NSP may prevent the endogenous enzymes from reaching important nutrients in the grain cells (White et al., 1981). Cell wall NSP in the digestive tract may form high molecular weight aggregates, which increase viscosity. Increased viscosity slows transit time, increases gelling properties of the digesta, and retards digestion and absorption, thereby causing depressed chick growth (Antoniou et al., 1981; Nasi, 1988; Marquardt et al., 1994). Demasking is the process where xylanases are able to break down water-insoluble-pentosans generally found in the cell walls of fibrous plant material (Brufau, 2006). Thereby, nutrients that are trapped within the cell wall matrices are liberated for use by the animal.

Wheat bran is commonly used as an ingredient in pig feeds. Wheat bran inclusion is restricted due to the detrimental effects of arabinoxylans on digestion and utilization of nutrients. Treating wheat bran with cell wall-degrading enzymes may increase its digestibility. The effects of cell wall-degrading enzymes on carbohydrate fractions and

metabolites in the stomach and ileum of pigs fed wheat bran based diets were investigated (Van der Meulen et al., 2001). In this trial, wheat bran was either pre-incubated with acetic acid, pre-treated with a cellulase and/or xylanase enzyme prior to diet preparation, or the final diets treated with the enzymes just before feeding to barrows fitted with stomach and post valve T-caecum cannulas. Incubation of wheat bran without enzymes reduced NDF and increased the amount of soluble carbohydrates. Enzyme incubation increased the amounts of soluble saccharides in the stomach and small intestine. Addition of xylanase reduced the amounts of soluble carbohydrates in the small intestine compared to cellulase. This was because the action of cellulase was not only limited to a primary attack on the cell wall, but also reduced the oligosaccharides to their monomeric compounds. Cell wall-degrading enzyme preparations may increase the amount of soluble saccharides in the stomach and small intestine and the ileal VFA concentrations (Van der Meulen et al., 2001).

Xylanase supplementation may affect apparent nutrient digestibility and endogenous N losses in pigs (Yin et al., 2000). Sites and extent of nutrient digestion of wheat and its by-products and their interactions with an exogenous NSP-degrading enzyme (xylanase) were studied. Four diets, based on wheat, recombined wheat, wheat plus bran, or wheat plus middlings, were used without or with xylanase addition. NSP content was highest in the diet based on wheat bran and lowest in diets based on wheat and recombined wheat. Dietary NSP from wheat reduced ileal and total tract digestibility, thereby increasing DM output, endogenous N secretion, and microbial fermentation. Xylanase supplementation increased total tract digestibility of DM, CP, and GE and ileal digestibility of DM, CP, and some AA (Thr, Val, Met, and Ile). Negative correlations

occurred for digestibility of DM, energy, CP, and AA with NSP content. The NSP contributed to a higher ileal flow of endogenous nitrogen and volatile fatty acids (VFA) and the proportion of DM fermented in the large intestine was increased. Generally, an increase in the amount of NSP in feed causes a reduction in digestibility in pigs, because pigs do not have the enzymes necessary to digest fibre. Supplemental xylanase will open up the cell walls and present entrapped nutrients to the pig's endogenous enzymes. Furthermore, a high NSP content of feed without corresponding supplementation with fibre-degrading enzymes increases the amount of DM in the large intestine. This will lead to fermentation by intestinal microbes thereby giving rise to a higher VFA production. In summary, cell wall-degrading enzyme preparations may increase the amount of soluble saccharides in the stomach and small intestine. Cell wall-degrading enzymes may also increase the volatile acid fermentation in the small intestine. An increase in amount of soluble saccharides in both the stomach and small intestine represents an improvement in overall energy utilization.

1.2.4.2 Phytase

Phosphorus from plants is not readily available to monogastrics because of phytate which is the major form of P in plants (NRC, 1998). Since monogastrics do not have the phytase enzyme (Brufau et al., 2006) most plant P is undigested in the GIT. Phytase supplementation has rarely been studied for wheat by-products in swine. An experiment was carried out to assess the effect of an *E.coli* phytase in a corn-soybean meal-based diet on growth performance, bone ash, and blood plasma concentration of P in starter and finisher pigs (Jendza et al., 2005). Phytase addition linearly improved ADG, G:F,

final body weight, and daily P absorption. In summary, *E.coli* phytase supplementation in the diet complemented the lack of endogenous phytase in the pig, thereby improving dietary availability of P. Supplemental phytase can thus help to prevent nutritional imbalances and resulting growth impairments.

The addition of phytase to diets containing wheat by-products has been studied in broilers. The influence of microbial phytase on apparent ileal AA digestibility of feedstuffs for 5-wk-old-broilers was investigated by Ravindran et al. (1999). Supplementation of microbial phytase (1200 FTU/kg) improved the digestibility of protein and AA in wheat middlings with a mean improvement of AA digestibility from 70.8 to 73.4%.

In summary, phytase addition to wheat by-product and corn-based diets for monogastric species can improve P, AA, and energy digestibility and also the DE contents of diets. Phytate binds essential nutrients thereby making them unavailable to monogastric animals. Supplemental phytase is able to hydrolyze phytate and release bound P and other minerals and make them available to the pig.

1.2.5 Summary

Information on the use of wheat by-products, particularly millrun and exogenous enzymes in growing finishing pig diets is limited and the results inconsistent. The nutrient profile of wheat millrun has not been well characterized. Being a by-product, the nutrient composition of millrun may be hard to predict accurately, and depending on its individual component streams, its chemical composition can vary greatly. The benefits and mode of action of enzymes in poultry nutrition has been well established,

mainly through viscosity reduction and demasking. In swine the benefits of exogenous enzymes is more of demasking and less of viscosity reduction (Brufau, 2006).

Following a review of the literature, a general hypothesis was developed: xylanase and phytase will improve nutrient digestibility of diets based on wheat by-products from dry milling. This hypothesis was tested in 4 studies. Each of the studies has specific hypotheses and research objectives, which are listed as follows:

Study 1 hypothesis: Nutrient digestibility of a diet containing wheat millrun is lower than that of a wheat-based diet and can be improved by the use of xylanase and phytase, resulting in equivalent nutrient digestibility and performance.

Study 1 objectives: 1) determine the linear and curvilinear effects of wheat millrun inclusion on the variables (a) ileal digestibility of energy, AA, P, and Ca, (b) total tract digestibility of energy, P, and Ca, and (c) growth performance; 2) determine the effects of xylanase and phytase supplementation in wheat millrun diets on these variables; and 3) compare the wheat control diet with the millrun diets supplemented with xylanase and phytase for these variables.

Study 2 hypothesis: Energy and amino acid digestibility of the individual by-product streams that constitute millrun is restricted by NSP and can be improved with xylanase.

Study 2 objectives: 1) to measure the variables digestibility and digestible content of GE, AA, P, and Ca of diets containing wheat, wheat millrun, and individual wheat by-products; 2) by difference, calculate these variables specifically for wheat millrun and by-products; and 3) to study the impact of xylanase supplementation on these variables and if xylanase interacts with diet and by-product streams.

Study 3 hypothesis: When included in diets based on millrun, xylanase and phytase either alone or in combination will affect the passage rate of digesta through the GIT of pigs.

Study 3 objectives: The objectives were: 1) to measure the effect of xylanase and phytase and their interaction on digestibility of energy, DM, AA, P, Ca, and DE content of diets containing millrun, and 2) to study the effect of xylanase and phytase and their interaction on the digesta passage rate and nutrient retention time of diets based on millrun.

Study 4 hypothesis: In millrun based diets, the site of NSP digestion in pigs can be influenced by xylanase and phytase individually or in combination. Wheat millrun when used with xylanase and phytase for weaned pigs will affect the site of NSP digestion.

Study 4 objectives: 1) to study the effect of xylanase and phytase and their interaction on the site of energy digestion in weaner pigs fed diets based on millrun, and 2) to study the effect of xylanase and phytase on the pH of the GIT.

2. EFECTS OF INDIVIDUAL OR COMBINED XYLANASE AND PHYTASE SUPPLEMENTATION ON ENERGY, AMINO ACID, AND PHOSPHORUS DIGESTIBILITY AND GROWTH PERFORMANCE OF GROWER PIGS FED WHEAT-BASED DIETS CONTAINING WHEAT MILLRUN

2.1 Abstract

The objective was to determine if dietary enzymes increase the digestibility of nutrients bound by non-starch polysaccharides (NSP), such as arabinoxylans, or phytate in wheat millrun. Effects of millrun inclusion rates (20 or 40%), xylanase (0 or 4,375 units/kg feed), and phytase (0 or 500 phytase units/kg feed) on nutrient digestibility and growth performance were investigated in a 2 x 2 x 2 factorial arrangement with a wheat control diet (0% millrun). Diets were formulated to contain 3.34 Mcal of DE/kg and 3.0 g of true ileal digestible Lys/Mcal of DE and contained 0.4% chromic oxide. Each of 18 cannulated pigs (36.2 ± 1.9 kg of body BW) was fed 3 diets at 3 x maintenance in successive 10-d periods for 6 observations per diet. Feces and ileal digesta were collected for 2 d. Ileal energy digestibility was reduced ($P < 0.01$) linearly by millrun and increased by xylanase ($P < 0.01$) and phytase ($P < 0.05$). Total tract energy digestibility was reduced linearly by millrun ($P < 0.01$) and increased by xylanase ($P < 0.01$). For 20% millrun, xylanase plus phytase improved DE content from 3.53 to 3.69 Mcal/kg of DM, a similar content to that of the wheat control diet (3.72 Mcal/kg of

DM). Millrun linearly reduced ($P < 0.01$) ileal digestibility of Lys, Thr, Met, Ile, and Val. Xylanase improved ($P < 0.05$) ileal digestibility of Ile and Val. Phytase improved ileal digestibility of Lys, Thr, Ile, and Val ($P < 0.05$). Millrun linearly reduced ($P < 0.05$) total tract P and Ca digestibility and retention. Phytase ($P < 0.01$) and xylanase ($P < 0.05$) improved total tract P digestibility, and phytase and xylanase tended to improve ($P < 0.10$) P retention. Phytase improved Ca digestibility ($P < 0.05$) and retention ($P < 0.01$). The 9 diets were also fed for 35 d to 8 individually housed pigs (36.2 ± 3.4 kg of BW) per diet. Millrun reduced ($P < 0.05$) ADFI, ADG, and final BW. Xylanase increased ($P < 0.05$) G:F; phytase reduced ($P < 0.05$) ADFI; and xylanase tended to reduce ($P = 0.07$) ADFI. In summary, millrun reduced energy, AA, P, and Ca digestibility and growth performance compared with the wheat control diet. Xylanase and phytase improved energy, AA, and P digestibility, indicating that non-starch polysaccharides and phytate limit nutrient digestibility in wheat by-products. The improvement by xylanase of energy digestibility coincided with improved G:F but did not translate into improved ADG.

2.2 Introduction

Dry milling of wheat removes much of the starch fraction in the grain to produce flour for human consumption and leaves wheat by-products as a residual (Holden and Zimmerman, 1991). Although wheat by-products generally have a higher content of non-starch polysaccharides (NSP), CP, and minerals than the parent wheat (Slominski et al., 2004), nutrients such as AA are digested to a lesser extent than in the parent grain (Sauer et al., 1977). Swine do not digest feedstuffs with a high NSP content well

(Barrera et al., 2004); therefore, the DE content of most grain by-products is low (NRC, 1998). In wheat by-products, P is partly bound as phytate-P (Garcia-Estepa et al., 1999). Pigs do not produce endogenous phytase (Golovan et al., 2001), and are therefore inefficient in hydrolyzing phytate (Pointillart et al., 1984), resulting in a low P digestibility in grains and their by-products (NRC, 1998).

Replacing conventional energy-providing feedstuffs such as wheat in swine diets with low-cost by-products can be attractive economically. The low nutrient digestibility caused by NSP and phytate in wheat by-products indicates that xylanase and phytase supplementation may increase nutrient utilization. The current study tests the hypothesis that the nutrient digestibility of wheat millrun diets is lower than that of a wheat diet and can be improved by using xylanase and phytase, resulting in equivalent nutrient digestibility and growth performance.

The objectives of the digestibility and growth performance studies were: 1) determine the linear and curvilinear effects of wheat millrun inclusion on the variables (a) ileal digestibility of energy, AA, P, and Ca, (b) total tract digestibility of energy, P, and Ca, and (c) growth performance; 2) determine the effects of xylanase and phytase supplementation in wheat millrun diets on these variables; and 3) compare the wheat control diet with the millrun diets supplemented with xylanase and phytase for these variables.

2.3 Materials and Methods

2.3.1 Experimental Design and Diets

Effects of millrun inclusion rates (20 or 40%), xylanase (0 or 4,375 units/kg of feed), and phytase (0 or 500 phytase units/kg of feed) were tested in a 2 x 2 x 2 factorial

arrangement in 8 wheat-based diets together with a wheat control diet (0% millrun), for a total of 9 diets in a fractional factorial arrangement. The xylanase was endo-1, 4-β-xylanase (EC 3.2.1.8; Porzyme 9300; Danisco Animal Nutrition, Marlborough, UK) and the phytase was 6-phytase (EC 3.3.26; Phyzyme XP; Danisco Animal Nutrition). The wheat millrun used for this study was steam pelleted (Dawn Foods, Saskatoon, Saskatchewan, Canada) to reduce bulk density and facilitate transport and was reground on a hammer mill across a 4-mm screen (New Life Feeds, Saskatoon, SK, Canada). The millrun contained the screenings, bran, and short fractions, but not the middlings fraction after flour milling of hard red spring wheat. A DE content of 2,900 kcal/kg (as-fed) and true digestible Lys content of 0.41% was assumed for wheat millrun used in the current study for diet formulations based on values for its individual components such as bran and shorts (NRC, 1998). Fourteen other wheat by-product samples were collected in western Canada for comparison.

The wheat control diet and wheat millrun diets were formulated to an identical digestible nutrient content (3.34 Mcal of DE/kg; 3.0 g true digestible Lys/Mcal of DE; Table 2-1) using canola oil and crystalline AA. In the diets, sodium bicarbonate was included together with salt to maintain Na and ensure that Cl concentration was not elevated because of L-Lysine HCl inclusion rates. Diets were formulated to be at requirement for digestible AA and marginally limiting in DE (by 60 kcal/kg), and were fortified to meet vitamin and mineral requirements (NRC, 1998). Xylanase and phytase were included at 167 and 100 g/metric ton of finished feed, respectively. Chromic oxide (0.4%) was added as an indigestible marker to diets.

2.3.2 Experimental Procedures

The animal protocols for the 2 studies were approved by the University of Saskatchewan Committee on Animal Care and Supply and followed established

Table 2-1 Ingredient and nutrient composition (as-fed basis) of the wheat control and 20 and 40% wheat millrun diets

Item	Wheat Control 0% Wheat millrun	20% Wheat millrun[1]	40% Wheat millrun[1]
Ingredient, %			
Wheat	83.26	61.83	40.26
Wheat millrun	-	20.00	40.00
Soybean meal	12.50	12.50	12.50
Canola oil	-	1.80	3.60
Dicalcium phosphate	1.20	0.70	0.40
Limestone	0.85	1.00	1.10
L-Lysine·HCl	0.49	0.47	0.45
Vitamin premix[2]	0.50	0.50	0.50
Mineral premix[3]	0.50	0.50	0.50
Sodium bicarbonate	0.29	0.29	0.29
Salt	0.20	0.20	0.20
L-Threonine	0.15	0.14	0.13
DL-Methionine	0.06	0.07	0.07
Calculated nutrient content[4]			
DE, Mcal/kg	3.34	3.34	3.34
True digestible Lys, g/Mcal DE[5]	3.0	3.0	3.0
Total phosphorus, %	0.60	0.60	0.60
Available phosphorus, %	0.41	0.31	0.25
Phytate phosphorus, %[6]	0.27	0.36	0.45
Calcium, %	0.70	0.70	0.70
Analyzed mineral content, %			
Total phosphorus	0.64	0.64	0.62
Total calcium	0.74	0.71	0.68
Analyzed substrate content, %			

Table 2-1 (continued) Ingredient and nutrient composition (as-fed basis) of the wheat control and 20 and 40% wheat millrun diets

Item	Wheat Control 0% Wheat millrun	20% Wheat millrun[1]	40% Wheat millrun[1]
Ingredient			
Non-starch polysaccharide			
Insoluble	6.49	9.31	13.24
Soluble	4.97	3.09	2.69
Analyzed substrate content, %			
Ingredient			
Non-starch polysaccharide			
Total	11.41	12.39	15.85
Arabinose			
Insoluble	1.41	1.94	2.47
Soluble	1.00	0.72	0.70
Total	2.38	2.66	3.16
Xylose			
Insoluble	2.15	3.28	4.86
Soluble	1.04	0.90	0.69
Total	3.17	4.18	5.56

[1]Xylanase was included at 167 g/1,000 kg of finished feed and phytase at 100 g/1,000 kg of finished feed to create the enzyme-supplemented diets.

[2]Provided per kg of diet: vitamin A, 8,250 IU; vitamin D_3, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine, 1 mg; d-biotin; 0.2 mg; and vitamin B_{12}, 0.025 mg.

[3]Provided per kg of diet: Zn, 100 mg as zinc sulfate; Fe, 80 mg as ferrous sulfate; Cu, 50 mg as copper sulfate; Mn, 25 mg as manganous sulfate; I, 0.5 mg as calcium iodate; and Se, 0.1 mg as sodium selenite.

[4]Calculated content: 0.33 % digestible Met; 0.56% digestible Thr; 0.26% digestible Cys; and 0.16% digestible Trp.

[5]Calculated content: 3.0 true digestible Lys/Mcal DE (0.94% apparent digestible Lys) and an ideal pattern of apparent digestible AA compared to Lys, i.e., Thr, 60 and Met, 30 (NRC, 1998).

[6]Calculated from analyzed phytate contents in wheat, millrun, and soybean meal (Haug and Lantzsch, 1983).

principles (CCAC, 1993). Two experiments were conducted at the Prairie Swine Centre Inc.

Exp. 1 (Digestibility Study). Eighteen crossbreed barrows (Camborough-22 x Line 65; PIC Canada Ltd., Airdrie, Alberta, Canada; initial BW, 36.2 ± 1.9 kg; initial age, 91 ± 7 d) were surgically fitted with a T-cannula at the distal ileum. Each pig was randomly fed 3 diets so that, in each period, each diet was fed to 2 out of 18 pigs to provide 6 observations per pig, for a total of 54 observations. Pigs were housed in individual metabolism pens (1.5 x 1.5 m) that allowed freedom of movement. Pens had plastic-coated expanded metal floors, polyvinyl chloride walls (0.9 m high) with plexi-glass windows (0.3 x 0.3 m), 1 single-space dry feeder, and 1 bowl drinker. Urine collection trays (1.5 x 1.5 m) were installed underneath pens during collection periods. Daily feed allowance was adjusted to 3 times maintenance (3 x 110 kcal of DE/kg of $BW^{0.75}$; NRC, 1998), which was fed in 2 equal meals at 0800 and 1600, resulting in an ADFI of 1.48, 1.68, and 1.94 kg/d during the first, second and third periods, respectively. Diets were fed as a wet mash, with water added to feed (approximately 1:1 wt/wt) immediately after adding feed to the feeder. Pigs had free access to water throughout the experiment. The three 10-d experimental periods consisted of a 6-d acclimation to the experimental diets, followed by a 2-d collection of feces and urine, and a 2-d collection of ileal digesta. Each experimental period was followed by feeding a regular production diet without antibiotics for 4 d for a total of 10 d between collections to avoid carry-over effects.

Urine and feces were collected for a minimum of 2 times per day at 0800 and 1600. Urine drained into a 4-L bottle containing 20 mL of 12 *N* HCl to prevent

volatilization of urinary N. Collected urine was weighed, and a 5% (by weight) subsample was filtered through cotton to remove solid particles. Digesta samples were collected for 2 d using bags containing diluted formic acid attached to the opened cannula barrel for 10 h. Feces were collected using plastic bags attached to the skin around the anus (Van Kleef et al., 1994). Collected digesta, feces and sub-sampled urine were pooled by pig and frozen at –20ºC. Prior to analyses, feces and digesta were thawed, homogenized, sub-sampled, and freeze-dried.

Exp. 2 (Performance Study). Seventy-two crossbreed pigs (36 barrows and 36 gilts; Camborough-22 x Line 65; PIC Canada Ltd.) with an initial BW of 36.2 ± 3.4 kg and initial age of 91 ± 7 d were used. Pigs were housed individually in 1 room and fed 1 experimental diet each for 35 d in 8 blocks to give 8 observations per diet. Within each block, barrows or gilts of equal BW were used. Pigs were selected within sex from a larger, single weaning group based on BW and assigned randomly within block and sex to 72 pens. The dimensions of the pens were 1.83 x 0.91 m. The flooring of the pen was fully slated concrete, and the siding was polyvinyl chloride planking. A single-space dry feeder was located at the front of the pen and a nipple drinker was located at the back of the pen. The room was maintained within the thermo-neutral zone for the pigs, with a 14 h light (0700 to 2100), 10-h dark cycle. The diets were provided ad libitum as a dry mash. Pigs had free access to water. The feeders were checked daily to ensure that the feed was flowing freely.

Pigs were weighed at the beginning of the experimental period (d 0), and weekly thereafter (d 7, 14, 21, 28, and 35). On each weigh day, feed disappearance was determined, and the combined data were used to calculate ADG, ADFI, and G:F.

2.3.3 Chemical Analyses

Feed and freeze-dried feces and digesta were ground finely in a Retch mill (model ZMI, Brinkman Instruments, Rexdale, ON, Canada) over a 1-mm screen and analyzed for DM by drying at 135°C in an airflow-type oven for 2 h (method 930.15; AOAC, 1990). Chromic oxide content of feed, feces, and digesta was analyzed by spectrophotometry (LKB-Ultraspec III model 80-2097-62; Pharmacia, Cambridge, UK) at 440 nm after ashing at 450°C overnight (Fenton and Fenton, 1979). The GE of feed, feces and digesta was analyzed by an adiabatic bomb calorimeter (Model 5003, Ika-Werke GMBH & Co. KG, Staufen, Germany); benzoic acid was used as a standard.

Feed, fecal, and digesta samples were analyzed for AA with pre-column derivation using phenylisothiocyanate (Guay et al., 2006). Norleucine was used as an internal marker and, following hydrolysis, the sample was dissolved in distilled water containing EDTA to chelate the metal ions. The Cys was determined as cysteic acid and Met as Met sulfone after preoxidation with performic acid and precolumn derivation using phenylisothiocyanate (Pierce Inc., Rockford, IL; Guay et al., 2006). Calcium and P in urine were determined using a Hitachi 912 analyzer (Zasoski and Burau, 1977). For Ca, a calorimetric endpoint determination method was used, and for P, an endpoint method with sample blanking method was used.

Phosphorus in feed, digesta, and fecal samples was analyzed by a spectrophotometer (Model 80-2097-62, LKB-Ultraspec III, Pharmacia) at 470 nm after ashing at 600°C (method 965.17; AOAC, 1990). The wheat by-product samples were analyzed for ADF (method 973.18; AOAC, 1990) and NDF (Van Soest et al., 1991) was analyzed using a fibre analyzer (Ankom 200, Ankom Technology Co., Fairport,

NY). Diet samples were analyzed for soluble and insoluble NSP and constituent sugars by GLC (Englyst and Hudson, 1987).

Based on the results of chemical analyses, apparent ileal digestibility of AA, total tract digestibility of Ca and P, ileal and total tract digestibility of GE and DM, and DE content were calculated using the Cr_2O_3 concentration of feed, digesta, and feces (Adeola, 2001). The ileal and total tract digestible Ca:P ratio was calculated. Daily Ca and P retention were calculated by determining daily Ca and P intake, and deducting daily excretion of Ca and P in feces (calculated via the determined apparent total tract fecal digestibility) and urine.

2.3.4 Statistical Analyses

For Exp. 1, differences in digestibility of energy, AA, Ca, P, and DM among diets were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). For Exp. 2, growth performance differences among diets were analyzed as a randomized complete block using the MIXED model procedure of SAS.

For Exp. 1 and 2, diets containing millrun were analyzed as a 2 x 2 x 2 factorial arrangement. The statistical model included the following effects: wheat millrun inclusion rate (20 and 40%), xylanase (with and without), and phytase (with and without), and all of their interaction terms. Period effect was not included in the statistical model because its impact was not significant. Linear and quadratic effects of millrun addition were determined using contrast statements for the 0, 20, and 40% control diets. In addition, the means of the wheat control diet and the 20 and 40% millrun diets with supplemental xylanase and phytase were separated by preplanned

comparisons. Individual pig was considered as the experimental unit. Differences were considered significant if $P < 0.05$.

2.4 Results

2.4.1 Nutrient Composition of Wheat By-Products

The collected wheat by-products samples varied widely in chemical composition (Appendix A). The millrun sample used for the present study was highest in ADF (overall range, 6.0 to 19.5% DM basis) and greater than average in NDF (41.7% DM basis; range, 19.6 to 42.4%). The high fibre content in this millrun sample increased the content of NSP such as arabinose and xylose in the 20 and 40% millrun diets (Table 2.1), thereby reflecting an increased content of arabinoxylans in diets containing millrun. Calculated phytate P content was also greater in diets containing millrun.

2.4.2 Energy and Dry Matter Digestibility

Ileal. Millrun inclusion linearly reduced ($P < 0.01$) energy digestibility from 77.5 to 62.0% and ileal DE content from 3.42 to 2.90 Mcal/kg of DM (Table 2.2). Xylanase and phytase independently improved ($P < 0.05$) energy digestibility and DE content in the millrun diets. Energy digestibility in xylanase and phytase-supplemented millrun diets did not approach the coefficient obtained with the wheat control diet. Xylanase and phytase together resulted in a similar DE content as the wheat control diet in the 20% millrun diets but not in the 40%-millrun diets ($P < 0.05$). Millrun inclusion linearly reduced ($P < 0.01$) ileal DM digestibility from 79.4 to 63.4%. Xylanase and phytase

Table 2-2. Effect of wheat millrun inclusion rate and xylanase and phytase supplementation to wheat millrun diets on apparent ileal and total tract energy and DM digestibility and DE content of diets fed to grower pigs in Exp. 1[1,2]

	Millrun, %										*P*-value					
	0	20				40				Pooled	Millrun[3]		Millrun diets[4]			
Item	wCon	Con	Xyl	Phy	Xyl + Phy	Con	Xyl	Phy	Xyl + Phy	SEM	L	Q	20 vs. 40%	Xyl	Phy	Xyl x Phy
Ileal digestibility																
Energy, %	77.5[a]	68.1	72.4	71.6	72.5[b]	62.0	68.1	67.4	66.6[c]	1.21	< 0.01	0.29	< 0.01	0.04	0.04	0.80
DE, Mcal/kg DM	3.42[a]	3.10	3.29	3.26	3.32[ab]	2.90	3.20	3.14	3.13[b]	0.06	< 0.01	0.40	< 0.01	0.01	0.03	1.00
DM, %	79.4[a]	69.9	73.9	73.8	74.2[b]	63.4	69.2	68.7	67.9[c]	1.09	< 0.01	0.27	< 0.01	0.01	0.01	0.69
Total tract digestibility																
Energy, %	84.4[a]	77.6	79.8	78.9	80.7[b]	71.5	75.5	73.4	73.1[c]	0.55	< 0.01	0.65	< 0.01	< 0.01	0.28	0.97
DE, Mcal/kg DM	3.72[a]	3.53	3.63	3.59	3.69[a]	3.34	3.55	3.43	3.43[b]	0.03	< 0.01	0.98	< 0.01	< 0.01	0.16	0.57
DM, %	86.7[a]	80.3	82.2	81.7	83.1[b]	74.2	77.9	76.1	75.9[c]	0.45	< 0.01	0.76	< 0.01	< 0.01	0.09	0.95

[a,b,c]Solely preplanned comparisons among the following 3 treatment means were made: wheat control and 0% millrun, xylanase plus phytase and 20% millrun, and xylanase plus phytase and 20% millrun. Superscripts are lacking if differences among these 3 means did not exist. Means within the same row with the same letter are not different ($P > 0.05$).

[1]Eighteen pigs (36.2 ± 1.9 kg) each fed 3 diets at 3 times maintenance in subsequent 10-d periods for 6 observations per diet. Treatment means are reported as least-squares means.

[2]Explanation of abbreviations: wCon, wheat control; Con, control; Xyl, xylanase; Phy, phytase; L, linear; and Q, quadratic.

[3]Linear and quadratic responses were analyzed using control diets containing 0, 20, and 40% millrun.

[4]Source of variation and probability only among the 8 diets that contain millrun.

independently improved ($P < 0.05$) ileal DM digestibility, but this improvement did not result in a coefficient that was equal to the wheat control diet ($P < 0.05$). Overall, xylanase and phytase did not interact to improve nutrient digestibility.

Total Tract. The inclusion of millrun linearly reduced total tract energy digestibility from 84.4 to 71.5% and total tract DE content from 3.72 to 3.34 Mcal/kg of DM ($P < 0.01$; Table 2.2). Xylanase, but not phytase, improved ($P < 0.01$) energy digestibility and the DE content in the millrun diets. For 20% millrun, xylanase plus phytase improved DE content from 3.53 to 3.69 Mcal/kg DM, a similar content as the wheat control diet (3.72 Mcal/kg of DM). Xylanase and phytase did not interact to improve energy digestibility. Millrun inclusion linearly reduced ($P < 0.01$) DM digestibility from 86.7 to 74.2%. Xylanase improved ($P < 0.01$) and phytase tended to improve ($P = 0.07$) DM digestibility in the millrun diets, but not to a coefficient equal to the wheat control diet ($P < 0.05$).

2.4.3 Ileal Amino Acid Digestibility

Millrun inclusion linearly reduced apparent digestibility of all AA (Table 2.3). Specifically, digestibility of Lys, Thr, Met, Cys, and Ile decreased by 7.8, 10.4, 7.1, 7.7, and 9.1%-units, respectively ($P < 0.01$). Within millrun diets, xylanase improved ($P < 0.05$) digestibility of Ile and Phe, and tended to improve ($P < 0.10$) digestibility of Leu, Thr and Tyr. Phytase supplementation improved ($P < 0.05$) ileal digestibility of Arg, His, Ile, Leu, Lys, Thr, Tyr, and Val and tended to improve ($P = 0.06$) Phe digestibility. Xylanase and phytase interacted to improve ($P < 0.05$) ileal digestibility of His.

Table 2-3 Effect of wheat millrun inclusion rate and xylanase and phytase supplementation to wheat millrun diets on apparent ileal amino acid digestibility of diets fed to grower pigs in Exp. 1[1,2]

	Millrun , %										*P*-value					
	0	20				40				Pooled	Millrun[3]		Millrun diets[4]			
Item	wCon	Con	Xyl	Phy	Xyl + Phy	Con	Xyl	Phy	Xyl + Phy	SEM	L	Q	20 vs. 40%	Xyl	Phy	Xyl x Phy
Apparent ileal amino acid digestibility, %																
Ala	73.9	67.9	66.3	66.7	69.5	58.1	63.7	67.9	70.6	2.64	< 0.01	0.56	0.18	0.22	0.02	0.10
Arg	85.5	83.3	83.9	85.2	85.4	82.4	85.8	85.9	84.7	0.96	0.02	0.60	0.71	0.25	0.03	0.89
Asp	83.3[a]	77.6	80.3	79.6	78.3[ab]	74.2	78.3	78.6	75.2[b]	1.65	< 0.01	0.57	0.05	0.67	0.79	0.09
Cys	80.5[a]	76.1	75.7	76.1	74.4[b]	72.8	74.6	72.9	74.9[b]	1.42	< 0.01	< 0.01	0.79	0.07	0.21	0.88
Glu	91.0[a]	88.5	89.9	89.6	90.5[ab]	85.6	88.7	87.9	87.6[b]	0.68	< 0.01	0.82	< 0.01	0.01	0.14	0.95
Gly	74.2	69.2	69.2	70.7	73.3	64.6	68.6	68.7	68.9	1.49	< 0.01	0.92	< 0.01	0.12	0.02	0.17
His	82.5	77.6	79.6	78.7	82.4	76.9	78.7	79.6	79.8	0.87	< 0.01	0.05	0.21	<0.01	< 0.01	0.01
Ile	83.8[a]	79.2	79.7	79.2	81.2[ab]	74.7	78.6	79.4	78.6[b]	0.97	< 0.01	0.99	< 0.01	0.05	0.03	0.43
Leu	84.6[a]	80.1	81.1	80.9	82.0[ab]	76.1	79.7	80.9	79.8[b]	0.93	< 0.01	0.85	< 0.01	0.09	0.01	0.73
Lys	86.4	82.0	82.9	82.7	83.5	78.6	82.6	83.7	82.4	1.03	< 0.01	0.72	0.19	0.13	0.04	0.73
Met	86.6[a]	81.5	79.9	84.7	80.8[ab]	79.5	79.9	81.5	79.2[b]	1.49	< 0.01	0.42	0.11	0.09	0.23	0.24
Phe	86.8[a]	81.2	83.4	83.4	83.2[b]	77.5	83.1	81.8	80.8[b]	0.74	< 0.01	0.35	< 0.01	<0.01	0.06	0.16
Pro	89.1[a]	84.9	85.7	85.8	86.9[ab]	80.9	83.9	84.9	84.5[b]	0.79	< 0.01	0.87	< 0.01	0.05	< 0.01	0.35
Ser	82.5	78.9	79.2	79.2	81.2	75.6	77.3	78.7	78.1	1.14	< 0.01	0.92	< 0.01	0.31	0.06	0.30
Thr	80.4[a]	74.9	75.1	76.2	77.9[ab]	70.0	73.9	75.4	75.3[b]	1.21	< 0.01	0.82	< 0.01	0.09	< 0.01	0.19
Tyr	85.5[a]	78.5	80.4	79.8	81.7[b]	75.0	77.9	79.6	78.5[b]	0.99	< 0.01	0.16	< 0.01	0.06	< 0.01	0.41
Val	82.5	76.1	77.1	76.9	78.7	72.1	74.3	76.9	76.2	1.24	< 0.01	0.43	< 0.01	0.23	0.01	0.31

[a,b]Solely preplanned comparisons among the following 3 treatment means were made: wheat control and 0% millrun, xylanase plus phytase and 20% millrun, and xylanase plus phytase and 20% millrun. Superscripts are lacking if differences among these 3 means did not exist. Means within the same row with the same letter are not different ($P > 0.05$).

[1]Treatment means are reported as least-squares means.

[2]Explanation of abbreviations: wCon, wheat control; Con, control; Xyl, xylanase; Phy, phytase; L, linear; and Q, quadratic.

[3]Linear and quadratic responses were analyzed using control diets containing 0, 20, and 40% millrun.

[4]Source of variation and probability only among the 8 diets that contain millrun

2.4.4 Phosphorus and Calcium Digestibility

Millrun addition linearly reduced ($P < 0.05$) ileal P digestibility from 53.8 to 34.8% and total tract P digestibility from 59.5 to 42.9% (Table 2.4). Xylanase and phytase supplementation tended to improve ($P < 0.10$) ileal P digestibility. Xylanase and phytase interacted to improve ($P < 0.01$) total tract P digestibility ($P = 0.05$ and $P < 0.01$, respectively) to coefficients similar statistically to that of the wheat control diet. For 20% millrun, xylanase plus phytase improved total tract P digestibility from 45.3 to 60.2%, a similar content as the wheat control diet (59.5%).

The inclusion of millrun reduced ileal and total tract digestible P content curvilinearly (linear, $P < 0.01$; quadratic, $P < 0.05$; Table 2.4). The minimum content seems to be 0.24 and 0.29 g/kg of DM for ileal and total tract digestible P, respectively. Xylanase ($P < 0.05$), but not phytase, improved ileal digestible P. Xylanase and phytase acted synergistically to improve ($P < 0.01$) total tract digestible P, resulting in a similar digestible P content as the wheat control diet in the enzyme-supplemented 20% millrun but not the 40% millrun diet ($P < 0.05$).

The addition of millrun linearly reduced ($P < 0.05$) ileal Ca digestibility from 62.5 to 45.1%, and total tract Ca digestibility from 61.6 to 45.2% (Table 2.4). Xylanase or phytase did not affect ileal Ca digestibility. Phytase, but not xylanase, improved ($P = 0.05$) total tract Ca digestibility. The addition of millrun linearly reduced ($P < 0.01$) ileal digestible Ca from 0.53 to 0.34 g/d and total tract digestible Ca from 0.52 to 0.34 g/d. Xylanase and phytase acted individually ($P < 0.05$) and also interacted to improve ($P < 0.01$) total tract digestible Ca content; however, the combined effects did not result in the contents equal to that of the wheat control diet.

Table 2-4 Effect of wheat millrun inclusion rate and xylanase and phytase supplementation to wheat millrun diets on apparent ileal and total tract phosphorus and calcium digestibility and digestible phosphorus and calcium contents of diets fed to grower pigs in Exp. 1[1,2]

	Millrun, %									*P*-value					
	0	20				40				Pooled	Millrun[3]		Millrun diets[4]		
Item	wCon	Con	Xyl	Phy	Xyl + Phy	Con	Xyl	Phy	Xyl + Phy	SEM	L	Q	20 vs. 40%	Xyl	Phy	Xyl x Phy
Ileal digestibility, %																
P	53.8	40.5	46.0	42.7	47.9	34.8	37.4	40.2	43.7	3.09	< 0.01	0.32	0.02	0.06	0.08	0.12
Ca	62.5	53.9	52.2	54.6	47.9	45.1	40.9	46.1	50.6	4.75	0.01	0.99	0.04	0.66	0.71	0.93
Ileal digestible minerals, g/kg DM																
P	0.38[a]	0.26	0.31	0.26	0.31[b]	0.24	0.25	0.26	0.29[b]	0.02	< 0.01	0.04	0.06	0.02	0.33	0.12
Ca	0.53[a]	0.42	0.38	0.43	0.33[b]	0.34	0.27	0.32	0.33[b]	0.03	< 0.01	0.89	< 0.01	0.03	0.92	0.49
Ca:P	1.35	1.65	1.23	1.67	1.05	1.42	1.12	1.21	1.15	0.08	0.58	0.02	< 0.01	< 0.01	0.16	< 0.01
Total tract digestibility, %																
P	59.5	45.3	47.5	51.9	60.2	42.9	44.4	45.9	53.5	3.45	0.01	0.18	0.07	0.05	< 0.01	< 0.01
Ca	61.6[a]	53.6	54.7	59.3	57.3[ab]	45.2	45.2	49.7	48.1[b]	2.81	< 0.01	0.95	< 0.01	0.74	0.05	0.85
Total tract digestible minerals, g/kg DM																
P	0.43[a]	0.29	0.31	0.32	0.39[ab]	0.29	0.29	0.29	0.35[b]	0.02	< 0.01	0.02	0.16	0.02	0.02	< 0.01
Ca	0.52[a]	0.43	0.38	0.47	0.37[b]	0.34	0.30	0.35	0.31[b]	0.02	< 0.01	0.02	0.16	0.02	0.02	< 0.01
Ca:P	1.22	1.45	1.21	1.49	1.03	1.19	1.03	2.17	1.0	0.38	0.96	0.59	0.92	0.05	0.52	0.12

[a,b]Solely preplanned comparisons among the following 3 treatment means were made: wheat control and 0% millrun, xylanase plus phytase and 20% millrun, and xylanase plus phytase and 20% millrun. Superscripts are lacking if differences among these 3 means did not exist. Means within the same row with the same letter are not different ($P > 0.05$).

[1]Treatment means are reported as least-squares means.

[2]Explanation of diets abbreviations: wCon, wheat control; Con, control; Xyl, xylanase; Phy, phytase; L, linear; and Q, quadratic.

[3]Linear and quadratic responses were analyzed using control diets containing 0, 20, and 40% millrun.

[4]Source of variation and probability only among the 8 diets that contain millrun

The addition of millrun increased the ileal digestible Ca:P ratio (quadratic, $P < 0.05$); the minimum value seems to be 1.12. Xylanase, but not phytase, reduced the ileal Ca:P ratio ($P < 0.01$) to a ratio similar to the wheat control diet. The total tract digestible Ca:P ratio was not affected by millrun, xylanase, or phytase.

2.4.5 P and Ca Retention

Daily intake of P was not affected by millrun inclusion or enzymes and was similar among diets (Table 2.5). Millrun linearly increased ($P < 0.05$) fecal P excretion from 4.9 to 6.6 g/d. Neither millrun nor enzyme affected urinary P excretion. Phytase, but not xylanase, reduced ($P < 0.01$) P excretion in millrun diet, and an interaction of phytase with xylanase reduced ($P < 0.01$) fecal P excretion further than solely with phytase. Millrun linearly reduced ($P = 0.01$) P retention from 7.3 to 4.9 g/d. Xylanase and phytase tended to improve P retention ($P < 0.10$). Phytase and xylanase interacted ($P < 0.05$) so that their combined effect resulted in P retention similar to that of the wheat control diet. For 20% millrun, xylanase plus phytase improved P retention from 4.9 to 6.5 g/d, a similar retention as the wheat control diet (7.3 g/d).

Millrun inclusion did not affect daily Ca intake (Table 2.5). Millrun tended to increase ($P = 0.05$) fecal Ca excretion. Phytase, but not xylanase, tended to reduce ($P = 0.09$) fecal Ca excretion. Millrun inclusion, phytase, and xylanase did not affect urinary Ca excretion. Millrun linearly reduced ($P < 0.01$) Ca retention from 7.8 to 5.9 g/d. Xylanase, but not phytase, improved ($P < 0.01$) Ca retention in the millrun diets.

Table 2-5 Effect of wheat millrun inclusion rate and xylanase and phytase supplementation to wheat millrun diets on phosphorus and calcium intake, excretion and retention of diets fed to grower pigs in Exp. 1[1,2]

	Millrun, %									*P*-value					
	0	20				40				Pooled	Millrun[3]		Millrun diets[4]			
Item	wCon	Con	Xyl	Phy	Xyl + Phy	Con	Xyl	Phy	Xyl + Phy	SEM	L	Q	20 vs. 40%	Xyl	Phy	Xyl x Phy
Phosphorus, g/d																
Intake	12.4	11.0	11.4	10.5	11.1	11.6	11.5	11.0	11.0	0.58	0.37	0.19	0.46	0.62	0.25	0.90
Urine	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.03	0.44	0.72	0.23	0.26	0.52	0.99
Feces	4.9	6.0	5.9	5.0	4.4	6.6	6.4	6.1	5.2	0.53	0.04	0.69	0.06	0.24	< 0.01	0.03
Excretion	5.1	6.1	6.1	5.1	4.5	6.7	6.6	6.2	5.3	0.54	0.04	0.71	0.06	0.27	< 0.01	0.03
Retention	7.3	4.9	5.3	5.4	6.5	4.9	4.9	4.8	5.7	0.48	0.01	0.05	0.21	0.07	0.09	0.02
Calcium, g/d																
Intake	13.1[a]	12.3	10.8	12.5	10.8[ab]	11.7	10.5	10.8	10.1[b]	0.58	0.11	0.91	0.06	< 0.01	0.52	0.16
Urine	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.06	0.41	0.75	0.24	0.28	0.48	0.98
Feces	5.0	5.7	4.9	5.1	4.6	6.4	5.7	5.5	5.3	0.46	0.05	0.96	0.06	0.11	0.09	0.39
Excretion	5.2	5.9	5.1	5.3	4.9	6.6	6.1	5.7	5.5	0.49	0.05	0.99	0.06	0.17	0.09	0.42
Retention	7.8[a]	6.5	5.7	7.2	5.9[b]	5.9	4.4	5.1	4.6[b]	0.35	< 0.01	0.85	< 0.01	< 0.01	0.19	0.24

[a,b]Solely preplanned comparisons among the following 3 treatment means were made: wheat control and 0% millrun, xylanase plus phytase and 20% millrun, and xylanase plus phytase and 20% millrun. Superscripts are lacking if differences among these 3 means did not exist. Means within the same row with the same letter are not different ($P > 0.05$).

[1]Treatment means are reported as least-squares means.

[2]Explanation of diets abbreviations: wCon, wheat control; Con, control; Xyl, xylanase; Phy, phytase; L, linear; and Q, quadratic.

[3]Linear and quadratic responses were analyzed using control diets containing 0, 20, and 40% millrun.
[4]Source of variation and probability only among the 8 diets that contain millrun.

2.4.6 Growth Performance

Millrun linearly reduced ($P < 0.01$) BW at all stages of the experiment (Table 2.6). On d 35, pigs fed 40% millrun diet were 7.9 kg lighter than pigs fed the wheat control diet ($P < 0.01$). Xylanase or phytase did not affect BW.

Millrun inclusion linearly reduced ($P < 0.05$) ADFI from 1.9 to 1.6 kg/d for d 0 to 7, and from 2.3 to 1.9 kg/d for d 8 to 14 (Table 2.6). Xylanase reduced ($P < 0.01$) ADFI from 2.7 to 2.5 kg/d for d 15 to 21, and tended to reduce ($P = 0.07$) ADFI from 2.5 to 2.4 kg/d for d 0 to 35. Phytase reduced ($P = 0.01$) ADFI from 2.9 to 2.5 kg/d for d 22 to 28 and from 3.3 to 2.9 kg/d for d 29 to 35, and reduced ($P < 0.05$) ADFI from 2.6 to 2.4 kg/d for d 0 to 35. Xylanase and phytase did not interact to affect ADFI.

Millrun linearly reduced ($P < 0.01$) ADG from 0.96 to 0.77 kg/d for d 0 to 7, from 1.23 to 1.02 kg/d for d 15 to 21 ($P < 0.01$), and tended to reduce ADG from 1.02 to 0.95 kg/d for d 0 to 35 ($P < 0.06$; Table 2.6). For d 0 to 35, xylanase or phytase did not affect ADG.

Millrun linearly reduced ($P < 0.01$) G:F from 0.46 to 0.37 for d 15 to 21 (Table 2.6), and xylanase tended to increase ($P = 0.05$) G:F. For d 0 to 35, millrun linearly reduced ($P = 0.05$) G:F and xylanase improved ($P < 0.05$) G:F from 0.38 to 0.40. Phytase did not affect G:F.

2.5 Discussion

In the current study, the inclusion of wheat millrun in diets for growing pigs reduced DE content, digestibility of energy, AA, Ca, and P; and growth performance. Individually or combined supplementation of xylanase and phytase in millrun diets

Table 2-6 Effect of wheat millrun inclusion rate and xylanase and phytase supplementation to wheat millrun diets on performance of grower pigs over time in Exp. 2[1,2]

	Millrun, %										P-value					
	0	20				40				Pooled	Millrun[3]		Millrun diets[4]			
Item	wCon	Con	Xyl	Phy	Xyl + Phy	Con	Xyl	Phy	Xyl + Phy	SEM	L	Q	20 vs. 40%	Xyl	Phy	Xyl x Phy
BW, kg																
d 7	42.3[a]	41.7	41.9	41.4	41.7[ab]	40.9	41.1	40.9	40.8[b]	0.36	< 0.01	0.77	< 0.01	0.50	0.40	0.80
d 14	53.5[a]	50.0	48.2	47.3	47.7[b]	47.3	46.6	47.3	46.9[b]	0.83	< 0.01	0.02	0.19	0.85	0.75	0.98
d 21	61.9[a]	55.7	55.1	54.7	55.0[b]	54.5	53.7	54.6	53.9[b]	0.98	< 0.01	0.04	0.08	0.80	0.51	0.70
d 28	68.4[a]	62.4	62.5	60.5	61.3[b]	60.8	60.1	60.8	60.5[b]	1.21	< 0.01	0.14	0.19	0.96	0.43	0.92
d 35	76.5[a]	70.4	70.1	67.4	68.7[b]	68.6	67.5	68.4	67.7[b]	1.45	< 0.01	0.23	0.29	0.86	0.30	0.94
ADFI, kg/d																
d 0 to 7	1.92	1.99	1.74	1.82	1.82	1.63	1.76	1.71	1.69	0.07	< 0.01	0.03	0.04	0.46	0.53	0.84
d 8 to 14	2.31[a]	2.25	2.06	2.06	1.98[b]	1.92	1.96	2.31	2.04[ab]	0.12	0.04	0.40	0.77	0.15	0.59	0.40
d 15 to 21	2.72[a]	2.64	2.57	2.57	2.38[b]	2.76	2.44	2.57	2.41[b]	0.10	0.77	0.39	0.98	0.01	0.08	0.09
d 22 to 28	2.84[a]	2.84	2.75	2.49	2.60[b]	2.92	2.69	2.49	2.49[b]	0.14	0.68	0.79	0.84	0.57	0.01	0.59
d 29 to 35	3.21[a]	3.20	3.07	2.84	2.94[ab]	3.33	3.12	2.95	2.78[b]	0.15	0.60	0.71	0.76	0.34	0.01	0.31
d 0 to 35	2.53	2.61	2.43	2.34	2.34	2.54	2.40	2.43	2.28	0.09	0.93	0.49	0.76	0.07	0.02	0.26
ADG, kg/ d																
d 0 to 7	0.96[a]	0.88	0.92	0.85	0.88[ab]	0.77	0.81	0.77	0.76[b]	0.07	< 0.01	0.77	< 0.01	0.45	0.43	0.77
d 8 to 14	0.90	0.89	0.90	0.85	0.86	0.90	0.77	0.91	0.87	0.05	0.94	0.84	0.78	0.31	0.85	0.87
d 15 to 21	1.23[a]	1.09	1.14	1.07	1.04[b]	1.02	1.01	1.03	0.99[b]	0.06	< 0.01	0.65	0.08	0.90	0.38	0.37
d 22 to 28	0.97	0.95	0.91	0.84	0.89	0.89	0.91	0.88	0.94	0.08	0.37	0.86	0.88	0.58	0.45	0.53
d 29 to 35	1.19[a]	1.13	1.08	0.99	1.07[ab]	1.10	1.06	1.08	1.03[b]	0.06	0.35	0.88	0.98	0.71	0.24	1.00
d 0 to 35	1.02[a]	0.99	0.99	0.91	0.95[ab]	0.95	0.92	0.94	0.92[b]	0.03	0.06	0.73	0.14	0.79	0.14	0.91
G:F																
d 0 to 7	0.49	0.45	0.53	0.47	0.49	0.46	0.46	0.44	0.46	0.02	0.26	0.20	0.10	0.07	0.53	0.95

Table 2-6 (continued) Effect of wheat millrun inclusion rate and xylanase and phytase supplementation to wheat millrun diets on performance of grower pigs over time in Exp. 2[1,2]

	Millrun, %										P-value					
	0	20				40				Pooled	Millrun[3]		Millrun diets[4]			
Item	wCon	Con	Xyl	Phy	Xyl + Phy	Con	Xyl	Phy	Xyl + Phy	SEM	L	Q	20 vs. 40%	Xyl	Phy	Xyl x Phy
G:F																
d 8 to 14	0.39	0.39	0.43	0.41	0.44	0.42	0.39	0.41	0.42	0.04	0.33	0.56	0.71	0.41	0.48	0.30
d 15 to 21	0.46	0.41	0.45	0.41	0.44	0.37	0.42	0.40	0.42	0.02	< 0.01	0.87	0.07	0.05	0.64	0.64
d 22 to 28	0.34	0.33	0.33	0.34	0.35	0.31	0.35	0.36	0.37	0.02	0.34	0.77	0.48	0.30	0.14	0.30
d 29 to 35	0.37	0.36	0.35	0.35	0.36	0.34	0.34	0.36	0.37	0.02	0.19	0.77	0.86	0.72	0.28	0.33
d 0 to 35	0.41	0.39	0.42	0.39	0.41	0.38	0.39	0.39	0.41	0.01	0.05	0.60	0.24	0.03	0.29	0.24

[a,b]Solely preplanned comparisons among the following 3 treatment means were made: wheat control and 0% millrun, xylanase plus phytase and 20% millrun, and xylanase plus phytase and 20% millrun. Superscripts are lacking if differences among these 3 means did not exist. Means within the same row with the same letter are not different ($P > 0.05$).

[1]Treatment means are reported as least-squares means.

[2]Explanation of diets abbreviations: wCon, wheat control; Con, control; Xyl, xylanase; Phy, phytase; L, linear; and Q, quadratic.

[3]Linear and quadratic responses were analyzed using control diets containing 0, 20, and 40% millrun.

[4]Source of variation and probability only among the 8 diets that contain millrun.

improved DE content and nutrient digestibility. Xylanase supplementation improved G:F, but neither xylanase nor phytase improved ADG.

2.5.1 Millrun Addition

Wheat by-products of dry milling for flour production include the following fractions: bran, shorts, screenings, and middlings (AAFCO, 1988). In western Canada, wheat millrun is produced by combining all or most of these fractions. The selected millrun sample contained the shorts, bran, and screenings fractions. The reduced DE content of millrun diets indicates that the actual DE content of millrun was lower than the assumed content of 2,900 kcal/kg (as-fed). The energy values of wheat by-products require, further definition.

The addition of wheat millrun to a wheat-based diet reduced energy, AA, and DM digestibility, likely due to an increased content of NSP, which pigs do not digest well (Barrera et al., 2004). The increased NSP content interferes with digestibility of other macronutrients thereby reducing energy digestibility (Bell et al., 1983). The NSP in wheat and thus wheat by-products are mostly arabinoxylans and cellulose (Zijlstra et al., 1999). These NSP encapsulate nutrients and can thereby act as a physical barrier to effective nutrient hydrolysis and absorption.

Wheat millrun inclusion reduced P and Ca digestibility and retention. The reduction is likely due to a combined effect of increased phytate content, anti-nutritional effects of NSP (Barrera et al., 2004), and the limited ability of pigs to digest phytate-P (Selle et al., 2000). Dietary Ca will be bound to phytic acid to form phytate, which renders the Ca unavailable to the pig.

Millrun inclusion reduced G:F, ADG, and final BW in the present study. Millrun inclusion did not affect ADFI, but the reduced DE content indicates that millrun inclusion reduced DE intake. The increase in NSP content in millrun diets might have prevented the expected increase in ADFI that may have compensated for the reduced DE content (NRC, 1998). Together with the simultaneous reduced G:F, the reduced ADG with millrun inclusion can thereby be explained.

2.5.2 Xylanase Supplementation

Xylanase supplementation of the millrun diets improved energy and DM digestibility and DE content in the present study. By-products of cereal grains have a high content of NSP (Slominski et al., 2004). Pigs do not produce the endogenous enzymes that are required to digest NSP; therefore, supplementation of NSP-degrading enzymes in high-NSP diets is one approach to reduce detrimental effects of NSP and improve the nutritional value for young pigs (Li et al., 1996). In diets containing wheat bran, NSP-degrading enzymes have been found to increase soluble saccharides in the stomach and small intestine and increased VFA in the ileum (Van der Meulen et al., 2001), indicating that NSP-degrading enzymes move NSP digestion partially from the large intestine to the small intestine. The NSP-degrading enzymes thus can improve energy utilization of high NSP diets in young pigs (Graham et al., 1986).

Xylanase improved apparent AA digestibility, similar to improved AA digestibility in wheat-based diets fed to grower pigs (Barrera et al., 2004), indicating that wheat NSP hamper AA digestibility. The arabinoxylans enclose AA in the grain,

thus directly interfering with AA digestion and absorption in the small intestine, or enhance secretion of endogenous AA.

Xylanase supplementation of the millrun diets improved ileal and total tract P digestibility. In mature cereal grains, a large portion of the P is present as phytate-bound P (Ravindran et al., 1994). The peripheral endosperm layers of wheat are major storage sites of phytate and P (Maga, 1982). These sites contain arabinoxylans, which are a major substrate for xylanase. The improved P digestibility might thus be an indirect benefit of xylanase, because P, either bound or not bound to phytate, would be better exposed to digestive enzymes or supplemental phytase.

The NSP-degrading enzymes have had inconsistent effects on growth performance in swine (Bedford and Schulze, 1998). For example, supplementation of a NSP-degrading enzyme to a wheat and canola meal based diet improved ADG as a result of improved ADFI (Zijlstra et al., 2004). Improved ADG has also been attributed to improved G:F (Bedford et al., 1992; Van Lunen and Schulze, 1996). In contrast, xylanase supplementation of millrun diets tended to reduce ADFI, improved overall G:F, and did not affect ADG in the present study. Xylanase supplementation was thereby unable to correct the lower final BW observed with millrun diets compared to the wheat control diet because the improved G:F was negated by a reduced ADFI.

Nutrient content and imbalances in feed can impact voluntary feed intake in pigs (Nyachoti et al., 2004). Energy content affects feed intake in most scenarios (NRC, 1998). Dietary CP and AA balance also influence feed intake (Henry et al., 1992). Apart from nutrients, physical capacity might also limit feed intake of pigs. In the present study, the millrun diets had a lower DE content than the wheat control diet, and were

bulkier. The physical feed intake capacity might explain the reduced ADFI during the first 2 wk of the present study. The release of nutrients with xylanase supplementation might reduce feed intake because of 2 reasons: (1) extra released nutrients might trigger a feedback mechanism to reduce feed intake as a result of a glucostatic or aminostatic response, and (2) a nutrient imbalance within the gastro-intestinal tract of the pig. Both scenarios would combine improved nutrient digestibility with a lowered ADFI, as was observed with xylanase supplementation.

2.5.3 Phytase Supplementation

Phytic acid is the major P storage compound of most seeds and cereal grains. In wheat, phytic acid is mostly contained in the bran, which contains 5% phytic acid (Garcia-Estepa et al., 1999). Wheat contains 0.32% phytate with approximately 87% of it contained in the aleurone layer, 13% in the germ, and 2% in the endosperm (O'Dell et al., 1972). Phytic acid can form complexes with multivalent cations such as Ca, Mg, Zn, and Fe, starch, free AA, and proteins (Selle et al., 2000), and thus exists in many forms. Most of the P in plant-based feedstuffs is present as phytate-P (Liao et al., 2005). Another form is phytin, which is the Ca and Mg salt of phytic acid (Oatway et al., 2001). Phytate-mineral complexes are generally insoluble at physiological pH (Ravindran et al., 1994), and bound minerals are thus unavailable to swine.

Phytase improved ileal and total tract DE contents and DM digestibility. Adding phytase to feeds containing phytate can catalyze the removal of the orthophosphate group from phytate (Maga, 1982), thereby releasing the bound nutrients and improving nutrient digestibility. Phytic acid binds the main energy macronutrient for swine, starch,

through hydrogen bonding (Oatway et al., 2001). Pigs have a limited ability to digest phytate-P because endogenous phytase necessary for hydrolysis of phytate is lacking (Golovan et al., 2001). Consequently, increasing the inclusion rate of wheat by-products in diets for swine will increase dietary phytic acid content and subsequently reduce energy, AA and P digestibility, as occurred in the present study.

Effects of phytase on energy digestibility of swine diets are rarely studied. Supplementing phytase to rice bran-based diets either low or high in phytate did not affect apparent ileal digestibility of GE in grower pigs (Liao et al., 2005). Dietary Ca and available P content of the experimental diets might affect phytase efficacy. The combination of a reduced Ca and P content and phytase supplementation increased nutrient and energy digestibility in diets for pigs (Johnston et al., 2004). The improved DE content with phytase in the present study indicates that complexes between phytic acid and macronutrients in the millrun are significant and that energy is less available without phytase supplementation.

The wheat millrun in the present study might have been low in intrinsic phytase activity. In cereals, intrinsic phytases are located primarily in the aleurone layers (Oatway et al., 2001), the outermost endosperm tissue of wheat, that contains protein bodies that store phytase. Logically, supplementation of exogenous phytase to the millrun-based diets should have had limited effect on nutrient digestibility because millrun should have significant intrinsic phytase activity. However, the millrun was steam-pelleted, which likely reduced or eliminated intrinsic phytase activity because wheat phytase is heat labile thereby explaining the positive response in nutrient digestibility to exogenous phytase.

The efficacy of phytase in improving the AA availability is still a matter of debate. Phytase supplementation improved apparent AA digestibility in some studies in swine (Mroz et al., 1994; Liao et al., 2005), whereas other studies reported a lack of improvements in protein or AA digestibility (Bruce and Sundstol, 1998; Traylor et al., 2001). The interaction between phytase and AA digestion might thus be multifaceted. In the present study, phytase supplementation improved apparent ileal digestibility of Lys, Thr, Val, Leu, and Ile. Phytate, thus, clearly binds AA in wheat millrun (Selle et al., 2000). Four possible complexes exist in pigs and poultry between phytin and protein that lower protein digestion (Selle et al., 2000; Kies et al., 2001). These include: 1) phytin-protein complexes in feedstuffs, 2) complexes between phytin and proteolytic enzymes, 3) de novo complexes between phytin and proteins during intestinal transit, and 4) de novo complexes between phytin and free AA during intestinal transit in the animal. Variations in these complexes may contribute to the conflicting results in the literature.

The effects of phytase on P digestibility or availability in plant-based feedstuffs have been well documented. In young pigs, phytase increased P availability (Yi et al., 1996), and an *E*. coli derived phytase improved Ca and P digestibility and retention (Adeola et al., 2004). In the present study, phytase supplementation to the millrun diets improved apparent P and Ca digestibility, indicating that both P and Ca are being liberated from phytate-P and phytin.

Phytase supplementation reduced ADFI and did not affect ADG and G:F in the present study. Pigs fed the millrun diets had a lower final BW than that of pigs fed the wheat control diet, and phytase did not improve final BW of pigs fed millrun diets.

Furthermore, supplemental phytase might have released nutrients, resulting in a dietary nutrient imbalance and reduced ADFI. The lack of increased ADG might be expected because the diets were not limiting in P, and excess dietary P does not increase ADG (Ekpe et al. 2002). In contrast, phytase supplementation to diets limiting in P improved ADG and G:F in young pigs (Adeola et al., 2004).

2.5.4 Xylanase and Phytase Interaction

Xylanase and phytase interacted positively on P digestibility in the present study, indicating a synergy between the 2 enzymes in hydrolyzing P. Specifically, apparent total P digestibility was improved 1.9-% units by xylanase, 4.8-% units by phytase, and 12.8-% units by the combination of xylanase and phytase, as opposed to the cumulative 6.7-% units. Enzyme synergy exists if the effect of the 2 enzymes combined is greater than the cumulative effect of each single enzyme. This synergy might exist because supplemental xylanase disrupts the cell wall matrix and hydrolyzes otherwise unavailable carbohydrates, while at the same time allowing the supplemental phytase to gain access to phytate-bound nutrients like P, proteins and starch (Oryschak et al., 2002). The synergy might also be caused by changes in digesta passage rate, thereby allowing a prolonged contact time of the phytase with its substrate at the optimum pH.

2.6 Implications

Wheat millrun has the potential to partially replace energy-yielding feedstuffs for grower-finisher pigs. The inclusion of wheat millrun into swine diets, however, reduced

digestibility of energy, AA and P, and reduced growth performance. Xylanase and phytase can partially ameliorate the reduced nutrient digestibility of diets containing millrun. The improved nutrient digestibility did coincide with improved G:F, but did not translate into an improved ADG.

3. EFFECTS OF XYLANASE SUPPLEMENTATION ON DIGESTIBILITY AND DIGESTIBLE CONTENT OF ENERGY, AMINO ACIDS, PHOSPHORUS, AND CALCIUM IN WHEAT BY-PRODUCTS FROM DRY MILLING IN GROWER PIGS

3.1 Abstract

Wheat by-products are opportunity feedstuffs that vary in nutritional value, partly due to arabinoxylans that limit nutrient digestibility. Millrun is a by-product from dry milling wheat into flour and contains varying amounts of the bran, middlings, screening, and shorts fractions. The digestible nutrient content of millrun is not well known. Effects of xylanase supplementation (0 or 4,000 units/kg feed) on energy, AA, P, and Ca digestibility were studied in a wheat control diet and 5 diets containing 30% by-product (millrun, middlings, shorts, screenings, and bran) in a 2 x 6 factorial arrangement. The wheat control diet was formulated to contain 3.34 Mcal DE/kg and 3.0 g true ileal digestible Lys/Mcal DE. Diets contained 0.4% chromic oxide. Each of 12 ileal-cannulated pigs (32.5 ± 2.5 kg) was fed 6 or 7 of 12 diets at 3 x maintenance in successive 10-d periods for 6 or 7 observations per diet. Feces and ileal digesta were each collected for 2 d. Diet affected ($P < 0.01$) and xylanase improved ($P < 0.05$) ileal and total tract energy and DM digestibility and also the DE content. Xylanase improved ($P < 0.05$) total tract energy digestibility of the millrun, shorts, and bran diets

by 6.8, 0.3, and 1.3 percent units respectively. Xylanase did not affect hindgut fermentation but reduced ($P < 0.05$) hindgut fermentable DE by 0.11 and 0.13 Mcal/kg DM in the millrun and bran diets, respectively. Diet affected ($P < 0.05$) and xylanase improved ($P < 0.05$) digestibility and digestible contents of AA in diets and by-products, including Lys, Thr, and Val. Xylanase improved ($P < 0.05$) Lys digestibility by 13.8, 5.0, 5.2, 6.0, and 14.1 %-units in millrun, middlings, shorts, screening, and bran, respectively. Diet affected ($P < 0.01$) total tract P and Ca digestibility. Xylanase increased ($P < 0.05$) digestible P and Ca content. In summary, nutrient digestibility varies among wheat by-product streams. Xylanase improved nutrient digestibility and DE content in wheat by-products; and the extent of improvement depended on the by-product. Xylanase supplementation may maximize opportunities to include wheat by-products in swine diets and ameliorate reductions in nutrient digestibility associated with arabinoxylans.

3.2 Introduction

Cereals such as wheat contain non-starch-polysaccharides (**NSP**) mainly in the cell wall (Diebold et al., 2004). Generally, wheat by-products have a higher NSP content than wheat (Slominski et al., 2004). Due to the NSP, nutrients contained in wheat by-products are not utilized well by swine (Sauer et al., 1977), because endogenous production of digestive enzymes that hydrolyze NSP is lacking (Barrera et al., 2003). The NSP and enclosed nutrients are therefore mostly digested in the large intestine via microbial fermentation (Li et al., 1996). Hindgut fermentation is less efficient for energy utilization than enzymatic hydrolysis in the small intestine (Noblet et al., 1994).

Dietary supplementation of exogenous enzymes such as xylanase may hydrolyze the main NSP of wheat, arabinoxylans, and thereby improve energy utilization by the pig (Diebold et al., 2004).

Wheat millrun is a by-product of the dry milling process of wheat into flour (Holden and Zimmerman, 1991) that generally combines the individual wheat by-product fractions bran, shorts, screening, and middlings (AAFCO, 1988) and contains around 9.5% crude fibre (Dale, 1996). Wheat millrun and by-product fractions are alternative feedstuffs for swine, and are readily available in Canada and the USA. However, the digestible nutrient profile of wheat millrun is not well characterized (Nortey et al., 2007).

The hypothesis of the present study is that beneficial effects of xylanase on energy, AA, and P digestibility differs among individual wheat by-product streams, wheat millrun, and wheat in grower pigs. The objectives were as follows: 1) to measure the variables digestibility and digestible content of GE, AA, P, and Ca of diets containing wheat, wheat millrun, and individual wheat by-products; 2) by difference, calculate these variables specifically for wheat millrun and by-products; and 3) to study the impact of xylanase supplementation on these variables and if xylanase interacts with diet and by-product streams.

3.3 Materials and Methods

3.3.1 Experimental Design and Diets

Effects of xylanase supplementation (0 or 4,000 units/kg feed) were studied in a wheat control diet and five 30%-by-product diets (millrun, middlings, shorts, screening, and

bran) in a 2 x 6 factorial arrangement, for a total of 12 diets. The xylanase was endo 1, 4-β-xylanase (EC 3.2.1.8; Porzyme 9300; Danisco Animal Nutrition, Marlborough, UK). The 5 by-products and wheat originated from a commercial flour mill (Dawn Foods, Saskatoon, Saskatchewan, Canada). Of the five wheat by-products, only the millrun used for this study was steam pelleted (Dawn Foods) to reduce bulk density and facilitate transport, and was reground on a hammer mill across a 4-mm screen (New Life Feeds, Saskatoon, Saskatchewan, Canada). The millrun contained the screening, bran, and short fractions, but not the middlings fraction after flour milling of hard red spring wheat.

The by-products can be described as follows. The contaminants that are separated from whole wheat seeds prior to flour milling are collectively called wheat screening, and typically consist of malformed wheat kernels, foreign seeds, and other contaminants. Generally, wheat screening contain less than 7% crude fibre, and not less than 35% broken or shrunken grain (Audren et al., 2002). The wheat bran is the coarse outer covering of the wheat kernel that is separated from cleaned and scoured wheat in the usual process of commercial flour milling and has about 12% crude fibre (AAFCO, 1988). Wheat shorts are the layer of the wheat kernel just inside the outer bran layer covering the endosperm (Huang et al., 1999), and usually contain 5 to 10% crude fibre and 15 to 20% CP. Wheat middlings consist mostly of fine particles of bran and germ and have at least 15% CP (O'Hearn and Easter, 1983). Wheat millrun consists of coarse bran, shorts, screening, and middlings (AAFCO, 1988) and contains around 9.5% crude fibre (Dale, 1996).The wheat control diet was formulated to contain 3.34 Mcal DE/kg and 3.0 g true ileal digestible Lys/Mcal DE (Table 3.1). In the wheat control diet,

$NaHCO_3$ was included together with salt to maintain Na and ensure that Cl concentration was not elevated because of L-Lys·HCl inclusion rates. The wheat control diet was formulated to be at requirement for digestible AA and marginally low in

Table 3-1 Ingredient and nutrient composition (as fed basis) of the wheat control diet

Item	Wheat control[1]
Ingredient, %	
Wheat	83.26
Soybean meal	12.50
Dicalcium phosphate	1.20
Limestone	0.85
Vitamin premix[2]	0.50
Mineral premix[3]	0.50
L-Lys·HCl	0.49
Sodium bicarbonate	0.29
Salt	0.20
L-Thr	0.15
DL-Met	0.06
Calculated nutrient content	
DE, Mcal/kg	3.34
True digestible Lys,[4] g/Mcal DE	3.0
Total P, %	0.60

Table 3-1 (continued) Ingredient and nutrient composition (as fed basis) of the wheat control diet

Item	Wheat control[1]
Available P, %	0.41
Ingredient, %	
Phytate P,[5] %	0.27
Ca, %	0.70

[1]The 5 wheat by-product diets were processed by mixing 70% of the wheat control diet with 30% of the wheat by-product. Subsequently, xylanase was included at 167 g/1,000 kg of finished feed to the wheat control and wheat by-product diets to create 12 diets in a 6 x 2 factorial arrangement.

[2]Provided the following per kilogram of diet: vitamin A, 8,250 IU; vitamin D_3, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg. folic acid, 2 mg; thiamine, 1 mg; D-biotin; 0.2 mg; and vitamin B_{12}, 0.025 mg.

[3]Provided the following per kilogram of diet: Zn, 100 mg as $ZnSO_4$; Fe, 80 mg as $FeSO_4$; Cu, 50 mg as $CuSO_4$; Mn, 25 mg as $MnSO_4$; I, 0.5 mg as $Ca(IO_3)_2$; and Se, 0.1 mg as Na_2SeO_3.

[4]Calculated content: 3.0 g true ileal digestible Lys/Mcal DE (0.94% apparent ileal digestible Lys) and an ideal pattern of apparent digestible AA compared to Lys, i.e. (as a % of Lys), Thr, 60 and Met, 30 (NRC, 1998).

[5]Calculated from analyzed phytate contents in wheat, millrun, and soybean meal (Haug and Lantzsch, 1983).

DE (by 60 kcal/kg), and was fortified to meet vitamin and mineral requirements (NRC, 1998). The by-product diets were produced by mixing the wheat control diet with 30% of the individual wheat by-products. Xylanase was included at 167 g/metric ton of finished feed reaching an activity of 4,000 Units/kg feed. Chromic oxide (0.4%) was added to the diets as an indigestible marker.

3.3.2 Experimental Procedures

The animal protocol was approved by the University of Saskatchewan Committee on Animal Care and Supply and followed established principles (CCAC, 1993). The experiment was conducted at the Prairie Swine Centre Inc.

Twelve crossbreed barrows (Camborough-22 x Line 65; PIC Canada Ltd., Airdrie, Alberta, Canada; initial BW, 32.5 ± 2.5 kg; initial age, 85 ± 7 d) were surgically fitted with a T-cannula at the distal ileum. Each pig was randomly fed 7 diets so that in each of 7 subsequent 10-d periods, each diet was fed to 1 pig. During the experiment, 2 pigs were removed, resulting in 7 observations for 3 diets and 6 observations for 9 diets, for a total of 75 observations.

Pigs were housed in individual metabolism pens (Nortey et al., 2007). Daily feed allowance was adjusted to 3 times maintenance (3 x 110 kcal DE/kg $BW^{0.75}$; NRC, 1998), which was fed in 2 equal meals at 0800 and 1600, resulting in an ADFI of 1.34, 1.47, 1.66, 1.82, 2.02, 2.24, and 2.45 kg/d during the first through to the seventh period, respectively. Diets were fed as a wet mash, with water added to the feed (approximately 1:1, wt/wt) immediately after adding feed to the feeder. Pigs had free access to water throughout the experiment. The seven 10-d experimental periods consisted of a 6-d acclimation to experimental diets, followed by a 2-d collection of feces and a 2-d collection of ileal digesta.

Feces were collected for a minimum of 2 times per day at 0800 and 1600. Digesta samples were collected for 2 d using bags containing diluted formic acid attached to the opened cannula barrel for 10 h. Feces were collected using plastic bags attached to the skin around the anus (Van Kleef et al., 1994). Collected digesta and feces were pooled

by pig and frozen at –20ºC. Prior to analyses, feces, and digesta were thawed, homogenized, subsampled, and freeze-dried.

Pigs were weighed at the start of the experimental period (d 0), and at the end of every period thereafter (d 10, 20, 30, 40, 50, and 60) to determine the maintenance requirements from which the daily feed allowance was calculated.

3.3.3 Chemical Analyses

Feed and freeze-dried feces and digesta were ground finely in a Retsch mill (model ZMI, Brinkman Instruments, Rexdale, Ontario, Canada) over a 1-mm screen and analyzed for DM by drying at 135ºC in an airflow-type oven for 2 h (method 930.15; AOAC, 1990). Chromic oxide content of feed, feces, and digesta was analyzed by spectrophotometry (model 80-2097-62, LKB-Ultraspec III, Pharmacia, Cambridge, UK) at 440 nm after ashing at 450ºC overnight (Fenton and Fenton, 1979). The GE of feed, feces, and digesta was analyzed with an adiabatic bomb calorimeter (model 5003, Ika-Werke GMBH & Co. KG, Staufen, Germany); benzoic acid was used as a standard.

Feed, feces, and digesta were analyzed for AA with precolumn derivation using phenylisothiocyanate (Guay et al., 2006). Norleucine was used as an internal marker and, following hydrolysis, the sample was dissolved in distilled water containing EDTA to chelate the metal ions. The Cys was determined as cysteic acid and Met as Met sulfone after preoxidation with performic acid and precolumn derivation using phenylisothiocyanate (Pierce Inc., Rockford, IL; Guay et al., 2006).

Phosphorus in wheat, by-products, feed, digesta, and feces was analyzed with a spectrophotometer (model 80-2097-62; LKB-Ultraspec III, Pharmacia) at 470 nm after

ashing at 600ºC (method 965.17; AOAC, 1990). The feed, wheat, by-products, digesta, and feces were analyzed for Ca by an atomic absorption spectrophotometer (method 985.01; AOAC, 1996). Following grinding over a 0.5-mm screen, diet and wheat by-product samples were analyzed for soluble and insoluble NSP and constituent sugars by GLC (Englyst and Hudson, 1987). Wheat and by-products were analyzed for ADF (method 973.18; AOAC 1990), and NDF (Van Soest et al., 1991).

Based on the results of the chemical analyses, apparent ileal digestibility of AA, total tract digestibility of Ca, and ileal and total-tract digestibility of P, GE, and DM, and DE content were calculated for the 12 diets using the Cr_2O_3 concentration of feed, digesta, and feces (Adeola, 2001). The digestibility coefficients and digestible nutrient content of the five wheat by-products were separated from the wheat control diet using the difference method (Fan and Sauer, 1995).

3.3.4 Statistical Analyses

Differences in digestibility of energy, AA, Ca, P, and DM among diets were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Diets and by-products were analyzed as a 2 x 6 or a 2 x 5 factorial arrangement, respectively. The statistical model for diets included the following effects: xylanase (with and without), wheat and by-product diets (6 levels), and their interaction terms, and initial BW as a covariate. The model for by-products contained 5 by-products instead of 6 diets. Treatment means were separated by the probability of difference in case an interaction or a trend for an interaction between the main factors

occurred. Individual pig was considered as the experimental unit. Differences were considered significant if $P < 0.05$ and were described as tendencies if $0.05 < P < 0.10$.

3.4 Results

3.4.1 Nutrient Composition of Wheat By-Products

The wheat by-product samples used for this study varied widely in fibre and NSP composition (Table 3.2). The wheat bran had the highest total NSP content (31.0% as fed; overall range 19.1 to 31.0%). The millrun sample had the highest insoluble NSP content (25.4%) and wheat had the lowest content (15.1%). The content of arabinose and xylose in by-products followed the observed total NSP content; however, the bran contained a relative higher percentage of soluble NSP. The analyzed nutrient composition of the by-product diets reflected the nutrient content of the wheat and wheat by-products (Table 3.3). The bran-based diet had the highest level of total NSP followed by the middlings, shorts, millrun, screening, and the wheat-control diet. Analyzed phytate-P content was highest in the bran-based diet, followed by the millrun-based diet, and was lowest in the shorts-based diet.

Table 3-2 Analyzed ADF and NDF and total, insoluble, and soluble NSP content of wheat and wheat by products

Item (% as fed)	Wheat	Millrun	Middlings	Screening	Shorts	Bran
ADF	2.8	16.8	7.8	11.5	9.5	12.0
NDF	10.9	38.9	25.4	22.3	29.5	37.9
NSP						
Arabinose						
Total	2.32	5.42	5.78	6.38	2.85	7.95
Insoluble	1.83	4.79	4.67	5.62	2.37	5.72
Soluble[1]	0.48	0.63	1.12	0.75	0.49	2.24
Xylose						
Total	3.32	10.15	8.05	9.06	6.46	12.45
Insoluble	2.70	9.47	6.40	7.84	5.87	8.99
Soluble	0.62	0.68	1.65	1.22	0.60	3.46
Mannose						
Total	0.19	0.27	0.64	0.28	0.22	0.26
Insoluble	0.16	0.23	0.57	0.24	0.20	0.22
Soluble	0.04	0.04	0.07	0.04	0.02	0.04
Glucose						
Total	0.19	9.68	7.09	6.94	11.50	9.40
Insoluble	0.16	10.26	5.74	6.43	11.85	7.93
Soluble	0.04	-0.58	1.35	0.51	-0.36	1.47
Galactose						
Total	0.41	0.67	0.70	0.63	0.64	0.84
Insoluble	0.19	0.54	0.38	0.50	0.45	0.54
Soluble	0.22	0.13	0.32	0.14	0.19	0.30
Non-starch polysaccharide						
Total	19.05	26.31	22.39	23.39	21.78	31.03
Insoluble	15.11	25.38	17.78	20.67	20.77	23.45
Soluble	3.94	0.93	4.61	2.72	1.01	7.58

Table 3-3 Analyzed nutrient composition (as fed basis) of the wheat control and wheat by-product diets

	Wheat	Wheat by-product				
Item	control	Millrun	Middlings	Shorts	Screening	Bran
Mineral content, %						
Total P	0.66	0.79	0.70	0.57	0.74	0.80
Phytate P	0.29	0.52	0.38	0.27	0.48	0.59
Total Ca	0.66	0.45	0.48	0.43	0.37	0.38
Non-starch polysaccharide content, %						
Insoluble	6.28	11.65	9.94	11.18	8.99	12.39
Soluble	1.77	1.85	2.79	1.23	2.12	2.83
Total	8.24	13.5	12.73	12.40	11.11	15.22
Arabinose						
Insoluble	1.47	2.81	2.44	2.81	1.84	3.11
Soluble	0.48	0.41	0.80	0.12	0.50	0.68
Total	1.95	3.21	3.24	2.93	2.34	3.79
Xylose						
Insoluble	2.16	4.31	3.69	4.25	3.09	4.74
Soluble	0.52	0.62	0.83	0.31	0.77	1.06
Total	2.68	4.94	4.53	4.56	3.86	5.80

[1]Soluble NSP is the difference between total and insoluble NSP.

3.4.2 Energy and Nutrient in Diets

Energy and DM Digestibility. Diet affected ($P < 0.01$; Table 3.4) and xylanase tended to improve ($P = 0.09$) ileal energy digestibility and DE content of the diets; diet and xylanase did not interact. Within diets, xylanase improved ($P < 0.10$) energy digestibility 7.1%-units for the millrun diet and 4.4%-unit for the bran diet. Diet affected ($P < 0.01$) and xylanase improved ($P < 0.05$) DM digestibility. Diet and xylanase tended to interact (P = 0.06) to affect totat tract energy digestibility. Diet and xylanase interacted (P < 0.06) to affect total tract DE and DM digestibility. Diet affected ($P < 0.001$) and xylanase improved ($P < 0.05$) total tract energy digestibility, DE content, and DM digestibility;. Within diets, xylanase improved total tract energy digestibility of the millrun, middlings, shorts, screening, and bran-based diets by 6.8, 0.7, 0.9, 1.1, and 1.3%-units, respectively. A strong relationship ($R^2 = 0.75$; Figure 3.1) existed between insoluble NSP content of the wheat, middling, shorts, screening, and bran-based diets and the uplift in total tract energy digestibility provided by xylanase; with the inclusion of the millrun diet the R^2 was 0.27. The R^2 for total and soluble NSP versus the uplift in energy digestibility for the 5 diets was 0.73 and 0.12, respectively (data not shown). For the millrun-based diet, xylanase improved ($P < 0.05$) the DE content by 0.22 Mcal/kg and DM digestibility ($P < 0.01$) by 4.7%-units. Xylanase did not affect the DE content of the wheat diet**.** Diet affected ($P < 0.01$) the energy fermented in the large intestine, whereas xylanase did not. The amount of DE fermented in the large intestine was highest for middlings and lowest for bran.

Table 3-4 Effect of wheat and wheat by-product diets and xylanase supplementation on ileal and total tract energy and DM digestibility and DE content in grower pigs[1]

Item	XYL[2]	Diet						Pooled SEM	*P*-value		
		Wheat	Millrun	Middlings	Shorts	Screening	Bran		Diet	XYL	Diet x XYL
Ileal digestibility											
Energy, %	-	71.9	66.6	63.1	65.4	65.2	69.0	2.25	<0.001	0.090	0.505
	+	71.8	73.7	61.9	67.0	66.5	73.4				
DE, Mcal/kg of DM	-	3.11	2.89	2.82	2.91	2.89	2.97	0.09	0.002	0.090	0.406
	+	3.12	3.19	2.75	2.98	2.92	3.25				
DM, %	-	71.9	61.7	61.2	63.4	63.3	65.1	2.30	<0.001	0.038	0.116
	+	69.7	69.9	61.7	64.5	65.0	71.7				
Total tract digestibility											
Energy, %	-	81.8^{a}	72.1d	75.7^{bcd}	76.2^{bcd}	78.0^{abc}	73.1^{cd}	1.23	<0.001	0.015	0.050
	+	81.5^{a}	78.9^{ab}	76.4^{bcd}	77.1^{abcd}	79.1^{ab}	74.4^{bcd}				
DE, Mcal/kg of DM	-	3.54^{a}	3.19^{d}	3.37^{abcd}	3.38^{abcd}	3.45^{abc}	3.25^{cd}	0.05	<0.001	0.026	0.031
	+	3.53a	3.51^{ab}	3.39^{abcd}	3.43^{abcd}	3.47^{abc}	3.29^{bcd}				
DM, %	-	81.5^{a}	71.5^{e}	74.6^{cde}	75.9^{bcde}	77.6^{abc}	72.1de	1.2 1	<0.001	0.003	<0.001
	+	81.1^{ab}	78.6^{abc}	75.8^{cde}	76.9^{abcd}	78.8^{abc}	73.6^{abc}				
DE fermented in large intestine[3]											
Mcal/kg of DM	-	0.42	0.40	0.55	0.47	0.69	0.34	0.12	0.071	0.906	0.934
	+	0.42	0.29	0.63	0.45	0.55	0.22				

[a-e]Means within the same item with the same letter are not different ($P > 0.05$).

[1]Twelve pigs (32.5 ± 2.5 kg) each fed 7 diets at 3x maintenance in subsequent 7 periods for 7 observations per diet. Treatment means are reported as least square means. XYL = xylanase.

[2]-, without xylanase; +, with xylanase.

[3]Calculated difference of total tract and ileal DE content

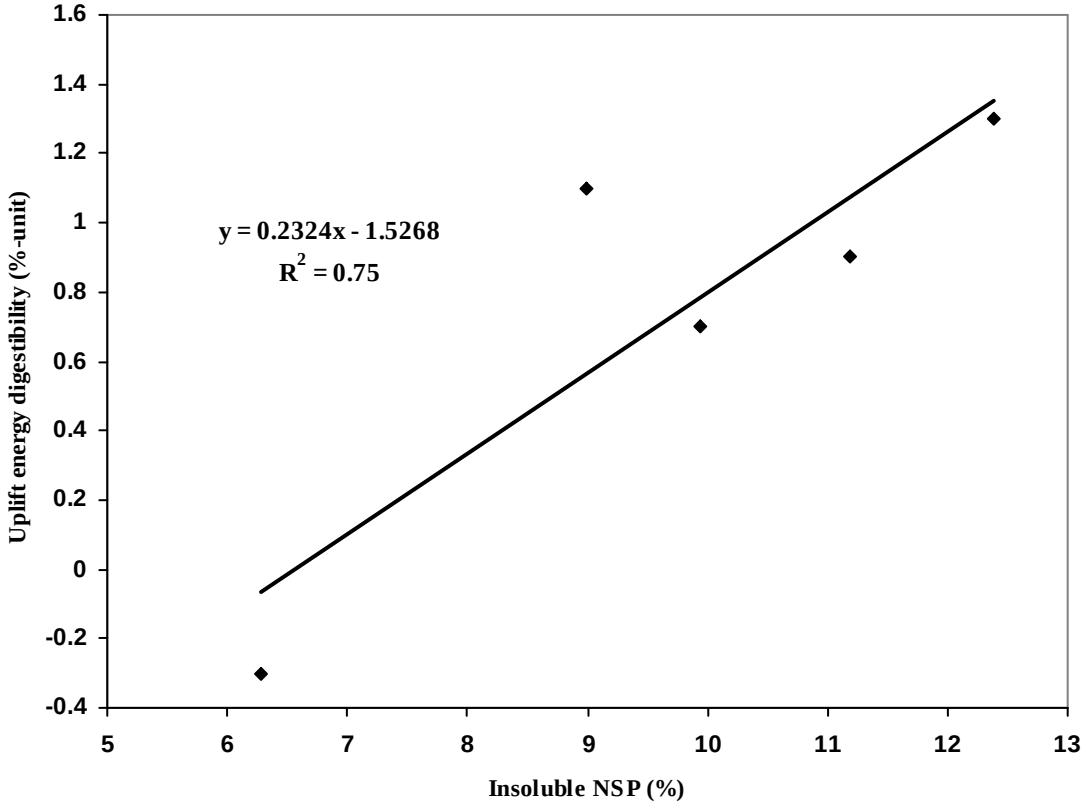

Figure 3-1 Relationship of the uplift in total tract energy digestibility (%-unit) provided by supplemental xylanase with the insoluble NSP content of five diets containing either strictly wheat or wheat and one of four wheat by-products (wheat millrun was excluded from the analysis).

Ileal AA Digestibility. Diet interacted with xylanase ($P < 0.05$; Table 3.5) to affect the AID of Ala, Arg, His, Leu, Lys, Phe, Thr, Tyr, and Val, and tended to interact ($P < 0.10$) for Cys, Gly, Ile, Met, and Ser. Xylanase improved ($P < 0.05$) the AID of Ala, Gly, Leu, Lys, Thr, and Val of the bran diet, but not for the other 5 diets. In the diets without xylanase, the AID of Lys was highest in the wheat diet and lowest in the middlings and bran diets.

Table 3-5 Effect of wheat and wheat by-products diets and xylanase supplementation on apparent ileal AA digestibility in grower pigs[1]

		Diet						Pooled	*P*-value		
Item	XYL[2]	Wheat	Millrun	Middlings	Shorts	Screening	Bran	SEM	Diet	XYL	Diet x XYL
Ileal AA digestibility, %											
Ala	-	70.4^{a}	61.5^{b}	64.9^{ab}	69.4^{ab}	66.5^{ab}	59.6^{c}	2.41	0.676	0.019	0.026
	+	67.4^{ab}	72.7^{a}	67.9^{ab}	65.9^{ab}	69.8^{ab}	70.0^{ab}				
Arg	-	83.7^{ab}	83.5^{ab}	82.5^{ab}	83.5^{ab}	82.5^{ab}	79.8^{b}	1.27	0.088	0.255	0.012
	+	79.7^{b}	87.5^{a}	82.0^{ab}	83.4^{ab}	83.4^{ab}	84.8^{ab}				
Asp	-	76.5	71.0	69.0	71.3	71.4	64.9	2.43	0.060	0.606	0.134
	+	72.7	75.6	66.5	71.0	69.8	72.7				
Cys	-	77.6^{a}	70.7^{abc}	66.2^{abc}	73.1^{abc}	65.7^{c}	61.5^{b}	2.56	<0.001	0.776	0.055
	+	74.0^{ab}	73.4^{abc}	62.9^{bc}	67.1^{abc}	68.8^{abc}	71.2^{ab}				
Glu	-	85.1	86.1	85.4	85.8	81.2	84.5	1.51	0.008	0.159	0.488
	+	87.1^{a}	87.9^{a}	85.1^{ab}	86.0^{ab}	83.3^{ab}	89.0^{ab}				
Gly	-	72.8^{a}	64.7^{bc}	59.1^{c}	66.6^{ab}	60.9^{bc}	59.8^{c}	2.73	0.085	0.645	0.065
	+	69.3^{ab}	68.8^{ab}	60.4^{c}	61.4^{c}	65.2^{bc}	66.9^{b}				
His	-	80.7^{ab}	77.3^{ab}	76.8^{ab}	79.1^{ab}	77.3^{ab}	73.8^{b}	1.62	0.352	0.211	0.009
	+	77.8^{ab}	82.7^{a}	76.4^{ab}	76.2^{ab}	77.4^{ab}	81.3^{ab}				
Ile	-	82.7	80.6	78.6	80.6	78.9	76.8	1.55	0.402	0.421	0.066
	+	80.1	82.6	79.1	78.2	79.8	82.9				

(Table 3-5 continued) Effect of wheat and wheat by-products diets and xylanase supplementation on apparent ileal AA digestibility in grower pigs[1]

		Diet						Pooled	*P*-value		
Item	XYL[2]	Wheat	Millrun	Middlings	Shorts	Screening	Bran	SEM	Diet	XYL	Diet x XYL
Leu	-	82.9^{ab}	80.6^{ab}	79.4^{ab}	80.8^{ab}	79.3^{ab}	76.5^{b}	1.44	0.317	0.160	0.017
	+	80.3^{ab}	83.9^{a}	79.5^{ab}	79.4^{ab}	79.9^{ab}	83.5^{a}				
Lys	-	86.8^{a}	81.3^{abcd}	78.9^{cd}	81.6^{abcd}	84.2^{abc}	76.9^{d}	1.20	<0.001	0.005	0.001
	+	84.3^{abc}	85.1^{ab}	80.7^{bcd}	81.9^{abcd}	84.3^{abc}	82.9^{abc}				
Met	-	82.9^{a}	77.4^{ab}	75.6^{ab}	75.0^{ab}	71.4^{b}	75.6^{ab}	2.31	0.011	0.205	0.064
	+	80.9^{ab}	78.8^{ab}	72.0^{ab}	78.7^{ab}	77.2^{ab}	80.3^{ab}				
Phe	-	83.7^{ab}	81.3^{ab}	80.0^{ab}	81.5^{ab}	79.4^{ab}	78.1^{b}	1.37	0.068	0.076	0.035
	+	81.7^{ab}	85.5^{a}	79.9^{ab}	81.3^{ab}	80.1^{ab}	84.2^{ab}				
Pro	-	83.8	81.4	78.1	84.4	81.8	79.6	2.53	0.233	0.681	0.376
	+	80.3	85.2	76.9	82.6	81.8	85.9				
Ser	-	76.8^{a}	73.2^{ab}	70.7^{ab}	72.9^{ab}	62.3^{b}	68.6^{ab}	2.75	0.045	0.164	0.059
	+	76.1^{a}	76.8^{a}	69.9^{ab}	69.5^{ab}	72.1^{ab}	76.6^{a}				
Thr	-	77.5^{a}	72.2^{ab}	67.2^{bc}	70.7^{abc}	76.5^{ab}	62.9^{c}	2.06	<0.001	0.188	0.005
	+	74.0^{ab}	76.6^{ab}	68.9^{abc}	69.9^{abc}	73.1^{ab}	73.5^{ab}				
Tyr	-	76.4^{b}	78.3^{ab}	74.9^{abc}	75.3^{abc}	77.7^{ab}	71.3^{bc}	1.72	0.014	0.073	0.010
	+	74.2^{bc}	82.3^{a}	74.4^{abc}	76.3^{ab}	76.8^{ab}	80.5^{ab}				
Val	-	79.2^{ab}	76.5^{abc}	73.7^{bc}	76.8^{abc}	76.0^{abc}	70.3^{c}	1.58	0.037	0.117	0.004
	+	75.7^{abc}	81.4^{a}	74.3^{abc}	75.0^{abc}	76.3^{abc}	78.7^{ab}				

[a-c]Means within the same item with the same letter are not different ($P > 0.05$).

[1]Twelve pigs (32.5 ± 2.5 kg) each fed 7 diets at 3x maintenance in subsequent 7 periods for 7 observations per diet.

Treatment means are reported as least square means. XYL = xylanase.
[2]-, without xylanase; +, with xylanase.

P and Ca Digestibility. Diet affected ($P < 0.01$; Table 3.6) ileal P digestibility, but xylanase did not. Diet and xylanase interacted to affect ($P < 0.05$) total tract P digestibility. In the diets without xylanase, the millrun diet had a lower ($P < 0.05$) total tract P digestibility than the wheat diet. Xylanase did not affect total tract P digestibility for any of the diets. Diet affected ($P < 0.01$) total tract Ca digestibility but xylanase did not. Diet and xylanase interacted ($P < 0.05$) to affect total tract digestible P content. Xylanase improved ($P < 0.05$) total tract P content for the millrun diet 0.12 g/kg DM, but not for any of the other diets. Diet affected ($P < 0.01$) total tract digestible Ca content, with the highest total tract digestible Ca content for the wheat diet.

3.4.3 Energy and Nutrients in Wheat By-products

Energy and DM Digestibility. By-product tended to affect ($P = 0.07$; Table 3.7) ileal energy digestibility and xylanase improved ($P < 0.01$) ileal DM digestibility. Diet and by-product tended to interact ($P = 0.09$) to affect total tract DE total tract energy digestibility. By-product tended to affect (P = 0.87) total tract DE content and affected the DM digestibility ($P < 0.05$). Xylanase improved ($P < 0.01$) total tract energy and DM digestibility and also the DE content of by-products. Within individual diets xylanase improved (P < 0.05) total tract DE content of the millrun, middlings, shorts, screening and bran diets by 0.96, 0.06, 0.20, 0.05, and 0.50 Mcal/kg of DM.,

Ileal AA digestibility. Xylanase increased ($P < 0.05$; Table 3.8) the AID of Arg, Ile, Leu, Lys, Phe, Ser, Thr, Tyr, and Val, and tended to increase the AID of Ala and His. By-product affected ($P < 0.05$) the AID of Glu, Lys, Pro, Ser, Thr, Tyr, and Val. Xylanase and by-product interacted ($P < 0.05$; Table 3.9) to affect the digestible AA

Table 3-6 Effect of wheat and wheat by-product diets and xylanase supplementation on ileal and total tract Ca and P digestibility and content in grower pigs[1]

Item	*YL*[2]	*Diet*						*Pooled SEM*	P-*value*		
		Wheat	Millrun	Middlings	Shorts	Screening	Bran		Diet	YL	Diet x YXL
Ileal digestibility, %											
P	-	51.7	30.9	29.8	34.4	39.8	52.9	4.5	0.003	(.155	0.131
	-	44.9	47.4	36.2	42.2	43.0	48.1				
Total tract digestibility, %											
P	-	52.4[a]	37.4[b]	43.8[ab]	42.1[ab]	46.9[ab]	47.8[ab]	2.8	0.029	(.222	0.045

										3	
	-	49.8[ab]	50.7[b]	41.5[ab]	46.0[b]	45.6[ab]	49.0[b]				
Ca	-	65.8	39.7	42.8	45.7	48.3	58.6	4.3	< 0.001	(. 5 2 4	0.178
	-	63.6	56.0	44.7	42.3	48.2	54.9				
Total tract digestible minerals, g/kg of DM											
P	-	0.39[abc]	0.32[c]	0.39[abc]	0.33[bc]	0.37[abc]	0.30[c]	0.02	0.009	(. 1 7 2	0.023
	-	0.37[abc]	0.44[a]	0.36[ab]	0.38[bc]	0.36[abc]	0.30[c]				

Ca	-	0	0	0.2	0	0.2	0	0	<	(	0.174
		.56	.19	1	.19	4	.28	.02	0.0	.	
									01	6	
										6	
										1	
	-	0	0	0.2	0	0.2	0				
		.54	.27		.18	4	.25				

[a-d]Means within the same item with the same letter are not different ($P > 0.05$).

[1]Twelve pigs (32.5 ± 2.5 kg) each fed 7 diets at 3x maintenance in subsequent 7 periods for 7 observations per diet. Treatment means are reported as least square means. XYL = xylanase.

[2]-, without xylanase; +, with xylanase.

Table 3-7 Effect of xylanase supplementation on the apparent ileal and total tract energy and dry matter digestibility coefficients and DE content of wheat by-products fed to grower pigs[1]

Item	*XYL*[2]	*By-product*					*Pooled*	P-*value*[3]		
		Millrun	Middlings	Shorts	Screening	Bran	SEM	By-product	XYL	By-product x XYL
Ileal digestibility										
Energy, %	-	51.4	41.8	50.3	49.4	62.7	7.90	0.066	0.220	0.647
	+	71.4	38.8	55.9	54.3	65.2				
DE, Mcal/kg of DM	-	2.42	2.09	2.43	2.35	2.61	0.35	0.122	0.205	0.582
	+	3.	1.8	2	2.4	3				

		35	9	.66	5	.01				
DM, %	-	37.1	35.3	42.9	43.4	49.7	7.73	0.296	0.005	0.404
	-	69.4	42.5	52.9	54.1	64.4				
Total tract digestibility										
Energy, %	-	55.8[b]	60.6[ab]	62.7[ab]	69.3[ab]	65.8[ab]	3.42	0.144	0.011	0.096
	-	75.8[a]	64.1[ab]	67.2[ab]	73.5[a]	66.4[ab]				
DE, Mcal/kg of DM	-	2.65	2.95	2.99	3.26	2.6	0.21	0.087	0.007	0.110

						1			5	
	-	3.56	3.01	3.19	3.31	3.11				
DM, %	-	54.9	59.0	62.9	68.6	43.2	4.43	0.013	< 0.001	0.156
	-	75.2	63.7	67.4	76.4	64.8				

[a-c]Means within the same item with the same letter are not different ($P > 0.05$).

[1]Twelve pigs (32.5 ± 2.5 kg) each fed 7 diets at 3 times maintenance in subsequent 7 periods for 7 observations per diet. Treatment means are reported as least square means. XYL = xylanase.

[2]-, without xylanase; +, with xylanase.

Table 3-8 Effect of xylanase supplementation on the apparent ileal amino acid digestibility of wheat by-products fed to grower pigs[1]

		By-product					Pooled	*P*-value		
Item	XYL[2]	Millrun	Middlings	Shorts	Screening	Bran	SEM	By-product	XYL	By-product x XYL
Ileal AA digestibility, %										
Ala	-	61.1	60.6	67.8	64.7	47.9	6.20	0.338	0.056	0.286
	+	78.4	61.2	61.9	71.9	66.8				
Arg	-	83.6	82.1	86.1	84.0	79.7	2.82	0.547	<0.001	0.213
	+	94.8	87.3	86.6	87.6	93.1				
Asp	-	61.5	51.9	64.9	59.6	50.3	8.44	0.242	0.298	0.679
	+	78.0	50.8	64.9	57.2	64.3				
Cys	-	55.4	46.1	62.8	51.9	41.9	6.6	0.115	0.166	0.663
	+	63.9	46.8	62.0	57.2	59.2				
Glu	-	81.5	80.1	82.3	72.6	84.4	4.81	0.032	0.256	0.819
	+	89.9	80.5	82.1	74.4	92.3				
Gly	-	61.3	37.1	55.7	52.2	42.3	8.96	0.203	0.222	0.908
	+	72.9	44.2	55.8	57.3	58.5				
His	-	76.6	70.0	75.9	73.4	71.1	4.69	0.149	0.057	0.369
	+	88.9	73.0	73.7	74.2	85.7				
Ile	-	77.1	74.1	75.4	70.3	71.4	5.45	0.630	0.044	0.818
	+	84.7	81.7	75.3	76.0	83.6				
Leu	-	78.4	75.4	75.8	77.1	73.3	4.2	0.399	0.020	0.398

Table 3-8 (continued) Effect of xylanase supplementation on the apparent ileal amino acid digestibility of wheat by-products fed to grower pigs[1]

Item	XYL[2]	By-product					Pooled	*P*-value		
		Millrun	Middlings	Shorts	Screening	Bran	SEM	By-product	XYL	By-product x XYL
Lys	+	89.6	77.7	77.4	79.2	88.4	3.50	0.002	<0.001	0.536
	-	73.1	67.2	71.1	78.2	62.7				
Met	+	86.9	72.2	76.3	84.2	76.8	6.51	0.167	0.422	0.979
	-	69.2	58.7	59.9	60.9	68.5				
Phe	+	74.	57.5	66.8	63.9	74.4	4.04	0.178	0.006	0.180
	-	75.5	73.3	78.5	75.0	72.9				
Pro	+	92.0	75.7	80.3	76.3	87.2	8.33	0.026	0.413	0.970
	-	85.1a	65.4	86.2	76.6	81.7				
Ser	+	94.2	65.5	87.4	78.9	92.4	5.60	0.047	0.035	0.393
	-	65.1	59.2	62.8	57.6	66.9				
Thr	+	80.6	62.1	60.7	65.2	82.2	6.05	0.001	0.020	0.445
	-	63.7	50.3	57.2	73.6	48.6				
Tyr	+	82.7	53.6	62.7	73.6	66.9	4.8	0.009	<0.001	0.013
	-	83.9^{abc}	73.0^{cd}	72.3^{c}	78.5^{bc}	60.0^{d}				
	+	97.9^{a}	74.2^{cd}	84.2^{abc}	81.6^{bc}	93.5^{ab}				

Table 3-8 (continued) Effect of xylanase supplementation on the apparent ileal amino acid digestibility of wheat by-products fed to grower pigs[1]

		By-product					Pooled	*P*-value		
Item	XYL[2]	Millrun	Middlings	Shorts	Screening	Bran	SEM	By-product	XYL	By-product x XYL
Val	-	72.9[bc]	65.4[bc]	73.2[bc]	73.9[b]	57.9[c]	4.72	0.010	0.019	0.062
	+	91.2[a]	63.9[bc]	71.2[bc]	74.9[b]	78.5[b]				

[a-d]Means within the same item with the same letter are not different ($P > 0.05$).

[1]Twelve pigs (32.5 ± 2.5 kg) each fed 7 diets at 3x maintenance in subsequent 7 periods for 7 observations per diet. Treatment means are reported as least square means. XYL = xylanase.

[2]-,without xylanase; +, with xylanase.

Table 3-9 Effect of xylanase supplementation on the apparent ileal digestible AA content of wheat by-products fed to grower pigs[1]

		By-product					Pooled	*P*-value		
Item	XYL[2]	Millrun	Middlings	Shorts	Screening	Bran	SEM	By-product	XYL	By-product x XYL
Ileal digestible AA, g/kg of DM										
Ala	-	0.57^{ab}	0.56^{ab}	0.68^{a}	0.53^{ab}	0.29^{c}	0.054	< 0.001	0.002	0.059
	+	0.77^{a}	0.72^{a}	0.60^{ab}	0.68^{a}	0.43^{bc}				
Arg	-	1.06^{cd}	0.84^{e}	1.12^{cd}	1.01^{d}	0.62^{f}	0.034	< 0.001	< 0.001	0.002
	+	1.45^{a}	1.19^{bc}	1.28^{b}	1.14^{c}	0.88^{e}				
Asp	-	0.50	0.55	0.86	0.62	0.45	0.100	0.007	0.118	0.277
	+	0.93	0.57	0.85	0.60	0.56				
Cys	-	0.22	0.22	0.20	0.10	0.07	0.104	< 0.001	0.435	0.162
	+	0.26	0.20	0.15	0.14	0.11				
Glu	-	3.02	2.34	3.36	1.91	3.07	0.241	< 0.001	0.735	0.898
	+	3.36	2.33	3.22	2.02	3.10				
Gly	-	0.59	0.37	0.62	0.37	0.31	0.067	< 0.001	0.019	0.662
	+	0.73	0.51	0.61	0.51	0.46				
His	-	0.51^{b}	0.37^{c}	0.49^{b}	0.41^{bc}	0.30^{d}	0.024	< 0.001	< 0.001	0.034
	+	0.58^{a}	0.49^{b}	0.47^{bc}	0.45^{bc}	0.42^{bc}				
Ile	-	0.48^{ab}	0.29^{c}	0.49^{ab}	0.51^{a}	0.38^{b}	0.029	< 0.001	< 0.001	0.006
	+	0.51	0.46	0.47	0.52^{a}	0.52^{a}				
Leu	-	0.89	0.67	0.95	0.81	0.69	0.059	0.002	0.001	0.201
	+	1.10^{a}	0.83^{b}	0.96^{ab}	0.86	0.93				

Table 3-9 (continued) Effect of xylanase supplementation on the apparent ileal digestible AA content of wheat by-products fed to grower pigs[1]

Item	XYL[2]	By-product					Pooled	*P*-value		
		Millrun	Middlings	Shorts	Screening	Bran	SEM	By-product	XYL	By-product x XYL
Lys	-	0.40^{d}	0.10^{f}	0.40^{d}	0.65^{bc}	0.20^{ef}	0.039	< 0.001	< 0.001	< 0.001
	+	0.75^{ab}	0.56^{c}	0.68^{bc}	0.84^{a}	0.30^{de}				
Met	-	0.19^{ab}	0.19^{ab}	0.16^{b}	0.17^{ab}	0.15^{b}	0.024	0.531	< 0.001	0.013
	+	0.24^{ab}	0.16^{b}	0.27^{a}	0.23^{ab}	0.27^{a}				
Phe	-	0.53	0.41	0.62	0.46	0.49	0.035	< 0.001	0.007	0.158
	+	0.72	0.46	0.64	0.46	0.56				
Pro	-	1.14	0.77	1.39	0.97	0.89	0.120	0.026	0.412	0.970
	+	1.54	0.89	1.39	1.03	1.29				
Ser	-	0.58^{ab}	0.43^{bc}	0.53^{b}	0.28^{c}	0.49^{b}	0.048	< 0.001	0.022	0.053
	+	0.75^{a}	0.47^{bc}	0.41^{bc}	0.44^{bc}	0.61^{ab}				
Thr	-	0.39	0.21	0.34	0.67	0.18	0.044	0.001	0.020	0.445
	+	0.60	0.43	0.54	0.63	0.40				
Tyr	-	0.45^{bc}	0.33^{d}	0.35^{d}	0.39^{d}	0.19^{e}	0.022	0.009	< 0.001	0.013
	+	0.64^{a}	0.49^{b}	0.52^{b}	0.46^{bc}	0.62^{a}				
Val	-	0.61^{b}	0.41^{c}	0.68^{b}	0.60^{b}	0.34^{c}	0.038	0.011	0.019	0.062
	+	0.87^{a}	0.55^{b}	0.67^{b}	0.63^{b}	0.52^{b}				

[a-d]Means within the same item with the same letter are not different (P > 0.05).

[1]Twelve pigs (32.5 ± 2.5 kg) each fed 7 diets at 3x maintenance in subsequent 7 periods for 7 observations per diet. Treatment means are reported as least square means. XYL = xylanase.

[2]-,without xylanase; +, with xylanase.

content of Arg, His, Ile, Lys, Met, and Tyr, and tended to interact ($P < 0.10$) for Ala and Ser.

P and Ca digestibility. By-products affected ($P < 0.01$; Table 3.10) total tract P and Ca digestibility. Xylanase did not affect total tract P and Ca digestibility, but increased ($P < 0.05$) digestible P content and tended to increase ($P < 0.10$) digestible Ca content.

3.5 Discussion

In the present study, including millrun and the individual by-product streams that together may form millrun in wheat-based diets for growing pigs reduced DE content, energy, DM, AA, P, and Ca digestibility. Supplemental xylanase did not affect the wheat-based diet, but improved the DE content and nutrient digestibility of the by-product diets. By difference, the digestible nutrient content and digestibility of the by-product either without or with xylanase was also calculated. Diet and by-product interacted with xylanase supplementation for some of the variables and millrun responded overall best to xylanase.

By-product Addition. The wheat by-products used in the present study result from the dry milling of wheat into flour for human consumption. With the exception of millrun, which was pelleted and then reground for feed preparation, all the other by-products were not processed further prior to feed manufacturing. The millrun was the same batch used in an earlier study (Nortey et al., 2007) and contained the shorts, bran, and screening fractions.

Table 3-10 Effect of xylanase supplementation on the apparent total tract P and Ca digestibility coefficients and digestible P and Ca content of wheat by-products fed to grower pigs[1]

Item	*XYL*[2]	*By-product*					*Pooled SEM*	P-*value*[3]		
		Millrun	Middlings	Shorts	Screening	Bran		By-product	XYL	By-product x XYL
Total tract digestibility										
P, %	-	32.9	36	45.7	44	30.2	6.428	0.008	0.161	0.989
	+	41.3	49	48.2	41	36.8	6.428			
Ca, %	-	26.0	12.2	15.5	34.2	5.3	3.316	<0.001	0.104	0.549
	+	37.7	16.3	23.7	39.5	9.5	3.316			

Total tract digestible minerals, g/kg of DM										
P	-	0.22	0.20	0.16	0.21	0.09	0.47	0.021		0.864
	-	0.46	0.21	0.25	0.36	0.39				
Ca	-	0.07	0.17	0.03	0.03	0.20[a]	0.13	0.57	0.059	0.579
	-	0.44	0.11	0.09	0.28	0.25				

[a-c]Means within the same item with the same letter are not different ($P > 0.05$).

[1]Twelve pigs (32.5 ± 2.5 kg) each fed 7 diets at 3 times maintenance in subsequent 7 periods for 7 observations per diet. Treatment means are reported as least square means. XYL = xylanase.

[2]-, without xylanase; +, with xylanase.

The inclusion of wheat by-products reduced DE content and energy, AA, and DM digestibility. The reduced digestibility is due to a higher fibre content in the diets, because by-products contain less starch and more NSP or fibre than the parent grain (Souffrant, 2001; Svihus and Gullord, 2002; Slominski et al., 2004). Pigs do not digest NSP and fibre well, because pigs lack the required endogenous enzymes (Barrera et al., 2004). Additionally, NSP can act as an anti-nutritional factor (Stanogias and Pearce, 1985), which can compromise the digestibility of other nutrients. The reduction in nutrient digestibility and DE contents varied among the different diets and individual by-product streams, likely variable due to the different chemical composition among the by-products (Table 3.1) including the ratios of soluble, insoluble, and total NSP content. By-product addition reduced ileal AA digestibility probably due to the increased NSP (Table 3.1) and phytate content. Wheat by-products have a higher content of protein (Slominski, et al., 2004) and AA (NRC, 1998) than the wheat grain. However, for the extra AA to be beneficial to the pig, the AA have to be available for hydrolysis by the endogenous enzymes within the porcine gastrointestinal tract (**GIT**). Nutrient digestibility is reduced by the presence of NSP (Bell et al., 1983) and phytate (Selle et al., 2000), because both can encapsulate or form complexes with important nutrients.

The proportion of total tract DE that occurred through hindgut fermentation differed among treatments, reflecting the different amount of fibre that cannot be hydrolyzed in the upper GIT. Hindgut fermentation results in VFA that are of less nutritional value for pigs than glucose (Noblet et al., 1994). For some of the diets, xylanase addition reduced the amount of hindgut fermentation, indicating that xylanase shifted the site of digestion

from the lower to the upper intestinal tract for these diets, thereby enhancing nutrient utilization.

By-product inclusion reduced P and Ca digestibility. Feedstuffs for swine are mainly of plant origin. The P that is found in plants is mainly in the form of phytate-P that is not readily available (Simons et al., 1990; Liao et al., 2005). Pigs do not produce endogenous enzymes within their GIT necessary to digest phytate P (Golovan et al., 2001). Increasing dietary inclusion of by-products and consequently phytate could thus reduce digestibility not only for P but also for other nutrients such as AA and minerals. The total tract digestible P was influenced by the type of by-product, reflecting the differences in digestibilities among the various by-products and their concentrations of available P via changes in the amount of total and phytate P. An increased amount of plant phytate P also means that more of the Ca present will be bound to phytate to form phytin, which is the Ca and Mg salt of phytic acid (Oatway et al., 2001). The reduction in digestible Ca content in by-product diets might be due to the combination of a higher NSP content in by-product diets, a reduced total dietary Ca, and a reduced ileal and total tract Ca digestibility. An increased NSP content might mean that more Ca is enclosed by the fibre matrix, making Ca less available to the pig.

Xylanase Supplementation. Xylanase improved energy and DM digestibility and DE content. In the present study, the greatest improvement in nutrient digestibility with xylanase occurred in the by-product diets whereas little improvement was observed for the diet solely based on wheat. The greater improvement for by-products might be due to a higher insoluble and total NSP content in the by-product diets, thereby presenting more substrate for the xylanase to hydrolyze, as evidenced by the strong relationship

between insoluble NSP and uplift in energy digestibility provided by xylanase. Cereal by-products have a higher NSP content than the parent grain (Slominski et al., 2004) and these can act as anti-nutritional factor by entrapping nutrients within the fibre matrix and thereby reducing digestibility. Arabinoxylans are the major fibre component of wheat (Zijlstra et al., 1999) and need to be completely or partially hydrolyzed so that the entrapped nutrients are released. Xylanase improved the nutritional value of high NSP diets by partial hydrolyzing soluble and insoluble NSP, decreasing digesta viscosity, and rupturing NSP-containing cell walls and thereby releasing their contents for enzymatic hydrolysis (Diebold et al., 2004). Xylanase randomly cuts the arabinoxylan backbone into small fragments and reduces the molecular weight (Tapingkae et al., 2007). Logically, a higher arabinoxylan content in a feed or feedstuff will increase the quantity of entrapped nutrients and thus provide a greater positive effect following xylanase supplementation.

The response of the diet based on millrun was consistently greater than either the wheat control or other by-product diets. One explanation might be that millrun was the sole by-product that had been steam-pelleted prior to feed mixing. Cereal grains contain endogenous enzymes including xylanase, and endogenous xylanase activity in steam-pelleted diets might be lower than in mash diets (Cowieson et al., 2005) due to heat induced xylanase inactivation. Pelleting wheat millrun in the present study might thus have inactivated endogenous xylanase activity, making millrun more responsive to xylanase supplementation. Furthermore, pelleting can alter the physiochemical properties of fibre making fibre more degradable with enzymes (Svihus et al., 2004).

Supplementing xylanase to the diets improved apparent ileal digestibility of selected AA, and total tract P digestibility within the individual diets, consistent with our earlier studies (Nortey et al., 2007). Xylanase also improved the apparent ileal AA digestibility of wheat-based diets in previous studies indicating that the digestibility of AA may be reduced by the wheat NSP (Barrera et al., 2004). Within individual diets, Ca digestibility was enhanced with xylanase, indicating that Ca is encapsulated within the fibre matrix of the wheat arabinoxylans. In digestibility studies, small uplifts in Ca digestibility following enzyme supplementation are difficult to observe in pigs fed diets formulated to Ca requirements, and that may be the cause for the lack of response (Nortey et al., 2007). In the present study, the wheat control diet was formulated to be at Ca requirement, and the by-product diets were Ca deficient by varying degrees. Any improvement in Ca digestibility would then be expected to be more pronounced in the by-product diets, as is evidenced in the present study.

The present study provides further evidence that xylanase supplementation increased P digestibility of wheat by-products (Nortey et al., 2007). Mature grains contain large amounts of phytate P, which is the storage form of plant P (Ravindran et al., 1994). Most of this phytate P is stored in the outermost layers of the seed, i. e., the peripheral endosperm layers (Maga, 1982) that also contain arabinoxylans. Arabinoxylans are a major substrate for xylanase, and an indirect benefit of adding xylanase to high-arabinoxylan diets is improved P digestibility. Small improvements in P digestibility with xylanase addition translate into improved digestible P values and P utilization.

3.5.1 By-product and Xylanase Interaction

The by-product by xylanase interaction on AA and P digestibility indicated that the extent of response to xylanase depended on the by-product. Complete hydrolysis of wheat arabinoxylans requires the presence of certain enzymatic activities including xylanases, β-xylosidase, α-arabinofuranosidase, and acetyl and feruloyl esterases (Debyser et al., 1997).The array of enzyme activities is necessary, because the linear backbone of arabinoxylans contains β-(1→4) linked D-xylopyranosol units to which α-arabinofuranosyl units are attached (Tapingkae et al., 2007). The by-products used for the present study contained different proportions of NSP, including arabinose, xylose, and galactose. Variations also existed among the by-products in the difference between total and soluble NSP, i.e., insoluble NSP. These factors may contribute to the different effects of xylanase on the by-products. Interestingly, millrun has the largest difference between total and soluble NSP. Not surprisingly, xylanase had the highest positive effect with millrun, because more substrate for xylanase in the form of insoluble NSP was present.

Cereal grains such as wheat, rye, and barley contain proteins that can inhibit xylanase efficacy (Debyser et al., 1999; Goesaert et al., 2003; Bonnin et al., 2005). Enzyme inhibition is a natural phenomenon that occurs in plant seeds to act as a defense mechanism and regulate plant metabolic processes. The presence of inhibitors can therefore negate effects that can be achieved by adding enzymes to a wheat-based diet for pigs. Most of the effects of endogenous xylanase inhibitors and xylanase have been studied in the food industry in bread making (Debyser et al., 1999); therefore non-inhibited xylanases have been developed to give uniform results in dough formation.

Another reason for different responses to xylanase supplementation might be varying levels of xylanase inhibitors in the various by-product fractions, thereby partly interfering with the action of added xylanase.

Nutrient digestibility in by-products followed a similar pattern to diets. Xylanase improved energy digestibility and DE content. The measured DE content of millrun, middlings, shorts, screening, and bran were 2.41, 2.09, 2.41, 2.36, and 2.65 Mcal/kg DM, respectively. The measured DE content for millrun in the present study was lower than assumed previously (2.90 kcal; Nortey et al., 2007), indicating the importance of feed quality analyses prior to feed mixing. Differences in observed AID of AA might be that the presence of fibre in diets increased the endogenous N losses from the pig (Schulze et al., 1995); however evidence for this hypothesis was not provided in the present study. A potential effect of fibre content on digesta passage rate and intestinal microbial activity will require further study.

Wheat by-products combined with exogenous xylanase can potentially replace energy-yielding feedstuffs in swine diets. However, the beneficial effects of xylanase on nutrient digestibility and digestible nutrient content are variable and depend on the by-product. Individual by-products have different fibre compositions that impact xylanase efficacy. Xylanase may provide a small or large positive effect depending on wheat and wheat by-product quality.

4. EFFECT OF INDIVIDUAL OR COMBINED XYLANASE AND PHYTASE SUPPLEMENTATION ON DIGESTA PASSAGE RATE AND DIGESTIBLE NUTRIENT CONTENT OF A DIET WITH REDUCED NUTRIENT SPECIFICATIONS CONTAINING WHEAT AND MILLRUN RELATIVE TO A POSITIVE CONTROL DIET IN GROWER PIGS

4.1 Abstract

Wheat millrun is a by-product from the dry milling of wheat into flour and is a potential feedstuff for swine. The nutritional value of diets containing wheat millrun can be enhanced by reducing the anti-nutritive effects of the arabinoxylan and phytate in wheat millrun, e.g., by using supplemental enzymes. In a 5 x 5 Latin square, effects of supplementing xylanase (4375 U/kg feed) and (or) phytase (500 FTU/kg feed) on energy, DM, and P digestibility and digesta passage rate and mean digesta retention time of a wheat-based diet containing 20% millrun were investigated in a 2 x 2 factorial arrangement together with a positive control diet. The positive control diet was formulated to 3.40 Mcal DE/kg, 2.69 g true digestible Lys/Mcal DE, 0.60% total P and 0.70% Ca. The content of Lys, P, and Ca was reduced by 10% and the DE content by 200 kcal/kg in the other 4 diets, including the un-supplemented negative control diet. Cannulated pigs (33.6 ± 1.9 kg) were fed at 3 x maintenance in 5 periods for 5 observations per diet. Feces and ileal digesta were each collected for 2 d. Xylanase and phytase interacted to increase ($P < 0.05$) ileal and total tract energy, DM, and Ca

digestibility and DE content of the negative control diet. Xylanase improved ($P < 0.01$) total tract energy and DM digestibility by 3.4 and 4.2%-units, respectively, and the DE content by 140 kcal/kg DM. Phytase improved total tract digestibility of P ($P < 0.05$) but not energy digestibility or DE content. Xylanase and phytase did not affect digestibility and digestible content of essential AA. Xylanase and phytase did not affect digesta passage rate and mean retention time. In conclusion, xylanase and phytase enhanced nutrient digestibility of a diet based on wheat and millrun with a reduced nutrient specification. The interactive effect of both enzymes together on improving digestibility and digestible nutrient content was lower than the combined effect of the two individual enzymes. Supplemental enzymes can be used to reduce anti-nutritive effects of arabinoxylans and phytate, and thereby enhance nutrient digestibility and reduce costs of swine diets containing wheat millrun.

4.2 Introduction

The use of wheat by-products may lower feed costs; however, these by-products contain non-starch-polysaccharides (NSP) that are not well utilized by swine. Similarly, most plant P is present as phytate P that is not readily digested by pigs (NRC, 1998). Pigs do not produce the required enzymes to hydrolyze NSP (Sauer et al., 1977; Diebold et al., 2004) or phytase to hydrolyze phytate and release P (Golovan et al., 2001). The NSP may entrap other nutrients such as AA, because protein and fibre are associated in feedstuffs (Schulze et al., 1994; Lenis et al., 1996), whereas phytate may also bind to other nutrients and energy (Selle et al., 2000). Supplemental fibre-degrading enzymes such as xylanase can hydrolyze NSP and supplemental phytase can hydrolyze phytate thereby making additional nutrients available to the pig (Barrera et al., 2004).

Transit time of digesta through the gastrointestinal tract (**GIT**) of pigs is affected by animal factor and feed characteristics (Stanogias and Pearce, 1985). Digesta transit time might explain the interaction between carbohydrase and phytase for nutrient digestibility (Oryschak et al. 2002). Feeding NSP to pigs may change digesta passage rate in pigs and thereby affect nutrient digestibility (Latymer et al., 1985; Owusu-Asiedu et al., 2006).

The hypothesis of the present study is that supplementing xylanase and phytase to a diet with a reduced nutrient specification will increase nutrient digestibility and thereby digestible nutrient content and may alter digesta passage rate. Using a diet with reduced nutrient content will clarify the uplift in nutrient digestibility provided by supplemental enzymes. The objectives were: 1) to measure the effect of xylanase and phytase and their interaction on digestibility of energy, DM, AA, P, Ca, and DE content of diets containing millrun, and 2) to study the effect of xylanase and phytase and their interaction on the digesta passage rate and nutrient retention time of diets based on millrun.

4.3 Materials and Methods

4.3.1 Experimental Design and Diets

Effects of xylanase supplementation (0 or 4,375 units/kg feed) and (or) phytase (0 or 500 FTU/kg feed) were studied in a 2 x 2 factorial arrangement in a diet with reduced nutrient specifications (negative control), together with a positive control diet, for a total of 5 diets in a fractional factorial arrangement. The negative control diet had been formulated to be low in energy by 200 kcal/kg, and in dietary Lys, Met, Thr, P, and Ca

by 10% (Table 4.1). The xylanase was endo-1, 4-β-xylanase (EC 3.2.1.8; Porzyme 9300; Danisco Animal Nutrition, Marlborough, UK), and the phytase was 6-phytase (EC 3.3.26; Phyzyme XP; Danisco Animal Nutrition). All diets contained 20% wheat millrun that had been steam-pelleted (Dawn Foods, Saskatoon, Saskatchewan, Canada) to reduce bulk density and facilitate transport and was reground on a hammer mill across a 4-mm screen (New-Life Feeds, Saskatoon, Saskatchewan, Canada). The millrun contained the screenings, bran, and short fractions but not the middlings fraction after flour milling of hard red spring wheat. The wheat control diet was formulated to contain 3.40 Mcal/kg DE and 2.8 g true ileal digestible lysine/Mcal DE. To the diets, 1% acid-insoluble ash was added to serve as marker for digestibility.

4.3.2 Experimental Procedures

The animal protocol for the study was approved by the University of Alberta Faculty Animal Policy and Welfare Committee (Protocol number ZIJL-2005-76), and followed established principles (CCAC, 1993). The experiment was conducted at the Swine Research and Technology Centre of the University of Alberta (Edmonton, Alberta, Canada).

Five crossbreed gilts (Large white x Duroc; Genex Hybrid; Hypor, Regina, Saskatchewan, Canada; initial BW, 33.6 ± 1.9 kg) were surgically fitted with a T-cannula at the distal ileum. The experiment was divided into 5 consecutive 14-d periods. Pigs were each randomly fed 5 diets in 5 separate periods for a total of 5 observations per diet in a 5 x 5 Latin square design. Pigs were housed in individual pens (7 x 5 x 4ft; L x W x H) that allowed freedom of movement.

Table 4-1. Ingredient and nutrient composition (as-fed basis) of the positive and negative control diets[1]

Item	Positive control	Negative control
Ingredients, %		
Wheat	58.60	65.05
Wheat millrun	20.00	20.00
Soybean meal	12.50	11.00
Canola oil	4.20	0.05
Limestone	1.29	1.25
Celite	1.00	1.00
Dicalcium phoshate	0.63	0.10
Vitamin premix[2]	0.50	0.50
Mineral premix[3]	0.50	0.50
L-Lys HCl	0.45	0.35
Salt	0.20	0.20
L-Thr	0.10	-
DL-Met	0.03	-
Calculated nutrient content[4]		
DE, Mcal/kg	3.40	3.20
ME, Mcal/kg	3.19	3.00
CP, %	17.41	17.37
True digestible Lys, g/Mcal DE[5]	2.69	2.55
Total Lys, %	1.09	0.99
True ileal digestible Lys, %	0.91	0.81
Total P, %	0.60	0.50
Available P, %	0.29	0.19
Ca, %	0.70	0.60
Calculated substrate content[4]		
Nonstarch polysaccharide % (total)	14.97	16.28
Arabinose	1.84	1.98
Xylose	3.97	4.21
Phytate	0.35	0.37
Analyzed mineral content, %		
Total P	0.71	0.63
Total Ca	0.82	0.74

[1]Xylanase was included at 167 g/1,000 kg of finished feed, and phytase was included at 100 g/1,000 kg of finished feed to create the enzyme-supplemented diets.

Table 4-1 (continued) Ingredient and nutrient composition (as-fed basis) of the positive and negative control diets[1]

[2]Provided the following per kilogram of diet: vitamin A, 8,250 IU; vitamin D_3, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine, 1 mg; D-biotin; 0.2 mg; and vitamin B_{12}, 0.025 mg.

[3]Provided the following per kilogram of diet: Zn, 100 mg as $ZnSO_4$; Fe, 80 mg as $FeSO_4$; Cu, 50 mg as $CuSO_4$; Mn, 25 mg as $MnSO_4$; I, 0.5 mg as $Ca(IO_3)_2$; and Se, 0.1 mg as Na_2SeO_3.

[4]Calculated content: positive control diet, 0.25% digestible Met, 0.50% digestible Thr, 0.26% digestible Cys, and 0.15% digestible Trp; negative control diet, 0.22% digestible Met, 0.40% digestible Thr, 0.26% digestible Cys, and 0.15% digestible Trp.

[5]Calculated content: positive control diet: 2.69 and negative control diet; 2.55 g true digestible Lys/Mcal of DE (0.91 and 0.81% apparent digestible Lys respectively) and an ideal pattern of apparent digestible AA compared to Lys [i.e., Thr, 60; and Met, 30 (NRC, 1998)].

Daily feed allowance was adjusted to 3 times maintenance (3 x 110 kcal DE/kg $BW^{0.75}$; NRC, 1998), which was fed in 2 equal meals at 0800 and 1600. Diets were fed as a wet mash, with water added to feed (approximately 1:1, wt/wt) immediately after adding feed to the feeder. Pigs had free access to water throughout the experiment. The 14-d experimental periods consisted of a 9-d acclimation to experimental diets, followed by a 2-d collection of feces and a 2-d collection of ileal digesta. Subsequently, on the morning of d 14, 1% Cr_2O_3 was mixed into to the morning ration prior to feeding. The time of first appearance of Cr_2O_3 at the terminal ileum was noted. Hourly samples of ileal digesta were collected into 200-g plastic containers for 8 h following time of first feeding and frozen immediately.

Feces were collected via grab sampling for a minimum of 2 times per day at 0800 and 1600. Digesta samples were collected for 2 d using bags containing diluted formic acid attached to the opened cannula barrel for 8 h. Collected digesta and feces were pooled by pig and frozen at –20ºC. Prior to analyses, feces and digesta were thawed, homogenized, sub-sampled, and freeze-dried. Pigs were weighed at the start of the experimental period (d 0), and at the end of each period thereafter to determine the maintenance requirements from which the daily feed allowance was calculated.

4.3.3 Chemical Analyses

Samples of all the major feed ingredients were taken prior to feed formulation. Feed and freeze-dried feces and digesta were ground finely through a 1-mm screen in a Thomas-Wiley Laboratory Mill (Arthur H. Thomas Co., Philadelphia, PA) and analyzed for DM by drying at 135ºC in an airflow-type oven for 2 h (method 930.15; AOAC, 1990). Chromic oxide content of feed, feces and digesta were analyzed using a spectrophotometer (Fenton and Fenton, 1979). Acid-insoluble ash content was analyzed by the method of Van Keulen and Young (1977). The GE of feed, feces, and digesta was analyzed adiabatically using an automatic bomb calorimeter (model AC-300, Leco Corporation, St. Joseph, MI).

Phosphorous in feed and feces were analyzed by a spectrophotometer (Model 80-2097-62; LKB-Ultraspec III, Pharmacia, Cambridge, UK) at 470 nm after ashing at 600ºC (method 965.17; AOAC, 1990). Calcium was analyzed by an atomic absorption spectrophotometer. Diet and digesta were analyzed for AA (Sedgwick et al., 1991). Here the separation and quantification of amino acids was accomplished with an HPLC and a Fluorichrom detector (excitation 340 nm; emission 450 nm). Samples were mixed, approximately 1:1 with the fluoraldehyde reagent immediately prior to injection and separations were achieved using a Supelcosil 3 micron LC-18 reverse phase column (4.6 x 150 mm; Supelco) equipped with a guard column (4.6 x 50 mm) packed with Supelco LC-18 reverse phase packing (20 - 40 μm). Diet and wheat by-product samples were analyzed for soluble and insoluble NSP and constituent sugars by GLC (Englyst and Hudson, 1987).

Based on the results of chemical analysis, total tract digestibility of Ca and P, and ileal and total-tract digestibility of GE and DM, and DE content were calculated using the acid-insoluble ash concentration of feed, digesta, and feces (Adeola, 2001). Digesta passage rate, mean retention time (MRT), and time of first appearance of Cr_2O_3 were determined using the formula: $\sum C_i t_i / \sum C_i$ (where C_i is the concentration of the maker at time t_i; Faichney, 1975).

4.3.4 Statistical Analyses

To compare the differences in digestibility of dry matter, energy, P, and Ca between the diets, data were analyzed by ANOVA using the GLM procedure within SAS as a 5 x 5 Latin Square Design. Main effects of xylanase, phytase, and their interaction term were determined in the diets with reduced nutrient specifications. The negative control diet was compared to the positive control diets using a contrast statement. Individual pig was considered as the experimental unit. Differences were considered significant if $P < 0.05$ and were described as tendencies if $0.05 < P < 0.10$. Treatment means were separated using the probability of difference solely for variables for which an interaction between the main effects was detected ($P < 0.10$).

4.4 Results

4.4.1 Energy and DM Digestibility

The ileal DE content was lower ($P < 0.05$; Table 4-2) for the negative control than the positive control diet. Xylanase and phytase interacted to increase ($P < 0.05$) the ileal

digestibility of energy and DM and the DE content of the negative control diet. Specifically, xylanase increased ($P < 0.05$) energy and DM digestibility by 8.8 and 9.9%-units, whereas phytase did not increase ileal energy and DM digestibility.

The total tract energy and DM digestibility and DE content was lower ($P < 0.01$; Table 4-2) for the negative control than the positive control diet. Xylanase and phytase interacted to increase ($P < 0.01$) these 3 variables of the negative control diet. Specifically, xylanase increased ($P < 0.05$) energy and DM digestibility by 3.4 and 4.2%-units and the DE content 0.14 Mcal/kg DM. Phytase increased ($P < 0.05$) DM digestibility but not energy digestibility.

4.4.2 Ileal AA Digestibility

The apparent ileal digestibility of Arg, Asp, His, Ile, Leu, Lys, Phe, Ser, Thr and Tyr was lower ($P < 0.05$; Table 4-3) for the negative control than the positive control diet.

Table 4-2. Effect of xylanase and phytase supplementation on apparent ileal and total tract energy and DM digestibility and digestible energy content of a diet with reduced nutrient specifications and a positive control diet fed to grower pigs[1]

Item	PCON	Reduced nutrient specifications				Pooled SEM	*P*-value[2]			
		NCON	XYL	PHY	XYL + PHY		PCON vs. NCON	XYL	PHY	XYL x PHY
Ileal digestibility, %										
Energy	67.0[ab]	60.9[b]	69.7[a]	67.4[ab]	65.1[ab]	2.49	0.10	0.21	0.71	0.04
DM	63.3[ab]	57.6[b]	67.5[a]	65.6[ab]	61.8[ab]	2.50	0.11	0.23	0.63	0.01
DE, Mcal/kg of DM	3.12[a]	2.71[b]	3.09[ab]	3.00[ab]	2.89[ab]	0.11	0.02	0.24	0.67	0.04
Total tract digestibility, %										
Energy	82.2[ab]	79.5[c]	82.9[a]	80.6[bc]	80.8[bc]	0.57	<0.01	<0.01	0.38	0.01
DM	80.5[ab]	77.7[c]	81.9[a]	79.4[b]	80.1[b]	0.48	<0.01	<0.01	0.82	0.01
DE, Mcal/kg of DM	3.82[a]	3.54[c]	3.68[b]	3.59[bc]	3.59[bc]	0.25	<0.01	<0.01	0.56	0.02

[a,b,c]Means within the same row with the same letter are not different ($P > 0.05$).

[1]Treatment means are reported as least squares means. Five pigs (32.6 ± 2.6 kg) each fed one of five diets at 3 times maintenance in subsequent 14-d periods for 5 observations per diet. PCON = positive control; NCON = negative control; XYL = xylanase; PHY = phytase.

[2]The *P*-values for XYL, PHY, and XYL x PHY are among the 4 diets with reduced nutrient specifications.

Table 4-3 Effect of xylanase and phytase supplementation on apparent ileal AA digestibility and digestible AA content of a diet with a reduced nutrient specifications and a positive control diet fed to grower pigs[1]

		Reduced nutrient specifications				Pooled	*P*-value[2]			
Item	PCON	NCON	XYL	PHY	XYL + PHY	SEM	PCON vs. NCON	XYL	PHY	XYL x PHY
Ileal AA digestibility, %										
Ala	76.4	64.4	73.5	74.9	71.5	6.72	0.24	0.69	0.54	0.38
Arg	85.0	80.2	81.0	84.1	81.9	1.28	0.01	0.59	0.08	0.24
Asp	74.2	68.6	69.8	72.8	69.0	1.99	0.05	0.55	0.42	0.24
Gly	64.4	58.6	61.3	63.9	59.6	2.71	0.13	0.77	0.51	0.22
Glu	85.1	81.9	83.3	84.6	82.4	1.43	0.11	0.77	0.56	0.23
His	85.2	80.1	80.6	84.5	82.0	1.29	0.01	0.48	0.04	0.26
Ile	80.5[a]	75.9[b]	77.8[ab]	79.8[ab]	76.3[ab]	1.52	0.04	0.63	0.47	0.09
Leu	81.8	77.2	78.8	80.6	78.5	1.46	0.03	0.86	0.33	0.24
Lys	86.0	79.9	79.9	82.9	80.3	0.02	<0.01	0.39	0.29	0.39
Phe	82.6	78.3	79.2	81.7	78.8	1.17	0.02	0.39	0.22	0.12
Ser	79.3	72.9	72.1	75.7	71.9	1.90	0.03	0.26	0.49	0.45
Thr	74.9	64.9	67.7	70.5	66.6	2.46	0.01	0.84	0.40	0.22
Tyr	78.8	72.2	72.8	76.9	74.1	2.10	0.04	0.62	0.17	0.43
Val	76.0	70.6	72.1	76.2	71.3	2.11	0.08	0.42	0.27	0.15
Digestible AA content, g/kg of DM										
Ala	0.58	0.48	0.54	0.55	0.53	0.05	0.19	0.69	0.53	0.39
Arg	0.82	0.76	0.76	0.77	0.78	0.01	<0.01	0.56	0.08	0.29
Asp	1.06	0.94	0.95	1.01	0.95	0.03	<0.01	0.37	0.24	0.17
Glu	3.42	3.26	3.32	3.37	3.28	0.06	0.06	0.77	0.57	0.24
Gly	0.51	0.46	0.48	0.51	0.47	0.02	0.09	0.81	0.46	0.23
His	0.35	0.33	0.33	0.34	0.34	0.001	<0.01	0.74	0.06	0.46
Ile	0.64	0.60	0.62	0.63	0.61	0.01	0.07	0.66	0.60	0.09

Table 4-3 (continued) Effect of xylanase and phytase supplementation on apparent ileal AA digestibility and digestible AA content of a diet with a reduced nutrient specifications and a positive control diet fed to grower pigs[1]

Item	PCON	Reduced nutrient specifications				Pooled SEM	*P*-value[2]			
		NCON	XYL	PHY	XYL + PHY		PCON vs. NCON	XYL	PHY	XYL x PHY
Digestible AA content, g/kg of DM										
Leu	1.10	1.02	1.05	1.07	1.04	0.02	<0.01	0.96	0.34	0.22
Lys	1.04	0.87	0.87	0.90	0.88	0.02	<0.01	0.42	0.33	0.42
Phe	0.76	0.70	0.71	0.73	0.71	0.01	<0.01	0.44	0.18	0,13
Ser	0.58	0.47	0.46	0.48	0.46	0.01	<0.01	0.32	0.55	0.55
Thr	0.49	0.37	0.39	0.40	0.38	0.02	<0.01	0.74	0.56	0.21
Tyr	0.31	0.27	0.27	0.29	0.28	<0.01	0.01	0.49	0.19	0.36
Val	0.76	0.69	0.70	0.74	0.69	0.02	0.02	0.42	0.31	0.18

[abc]Means within the same row with the same letter are not significantly different ($P > 0.05$).

[1]Treatment means are reported as least-square means. Five pigs (32.6 ± 2.6 kg) each fed 1 of 5 diets at 3 x maintenance in subsequent 5 periods for 5 observations per diet. PCON = positive control; NCON = negative control; XYL = xylanase; PHY = phytase.

[2]The *P*-values for XYL, PHY, and XYL x PHY are among the 4 diets with reduced nutrient specifications.

Xylanase and phytase did not affect the apparent ileal digestibility of the essential amino acids Lys and Thr. Phytase increased ($P < 0.05$) His digestibility and tended to increase ($P < 0.10$) Arg digestibility.

The ileal digestible content of Arg, Asp, His, Leu, Lys, Phe, Ser, Thr, Tyr, and Val was lower ($P < 0.05$;Table 4-3) for the negative control than the positive control diet, with a similar trend ($P < 0.10$) for Glu, Gly, and Ile. Xylanase and phytase did not affect the ileal digestible content of Lys and Thr. Xylanase and phytase interacted and tended to improve ($P < 0.10$) digestible content of Ile by 0.01 g/kg DM. Phytase tended to improve ($P < 0.10$) digestible content of Arg and His by 0.01 g/kg each respectively.

4.4.3 P and Ca Digestibility

The ileal digestibility and digestible content of Ca but not P was lower ($P < 0.05$; Table 4-4) for the negative control than the positive control diet. Xylanase and phytase interacted to increase ileal digestibility and digestible content of Ca ($P < 0.01$). Specifically, xylanase and phytase individually increased ($P < 0.05$) ileal digestibility and digestible content of Ca, but their combined effect was similar to their individual effect.

The total tract digestibility of P and digestible content of P and Ca was lower ($P < 0.05$; Table 4-4) for the negative control than the positive control diet. Xylanase and phytase increased ($P < 0.05$) total tract P digestibility, and increased ($P < 0.01$) or tended to increase ($P < 0.10$), respectively, the digestible content of P. Xylanase and phytase interacted to improve total tract digestibility and digestible content of Ca ($P < 0.01$). Specifically, xylanase and phytase individually increased ($P < 0.05$) total tract

Table 4-4. Effect of xylanase and phytase supplementation on apparent ileal and total tract P and Ca digestibility of a diet with reduced nutrient specifications and a positive control diet fed to grower pigs[1]

Item	PCON	Reduced nutrient specifications				Pooled SEM	*P*-value[2]			
		NCON	XYL	PHY	XYL + PHY		PCON vs. NCON	XYL	PHY	XYL x PHY
Ileal digestibility, %										
P	34.7	29.6	41.6	39.9	43.8	4.43	0.43	0.09	0.17	0.37
Ca	28.4^{ab}	21.1^{b}	39.6^{a}	38.6^{a}	25.4^{ab}	5.49	0.44	0.65	0.78	0.01
Ileal digestible nutrients										
P, g/kg of DM	0.27	0.21	0.31	0.29	0.32	0.03	0.26	0.07	0.19	0.34
Ca, g/kg of DM	0.26^{ab}	0.17^{b}	0.35^{ab}	0.36^{a}	0.22^{b}	0.05	0.29	0.70	0.65	<0.01
Total tract digestibility, %										
P	53.7	44.9	54.7	53.3	57.0	2.51	0.02	0.01	0.04	0.24
Ca	36.6^{ab}	28.7^{b}	44.0^{a}	46.8^{a}	34.7^{ab}	3.68	0.14	0.67	0.24	<0.01
Total tract digestible nutrients										
P, g/kg of DM	0.42	0.32	0.41	0.39	0.42	0.02	<0.01	<0.01	0.05	0.14
Ca, g/kg of DM	0.34^{abc}	0.24^{c}	0.39^{ab}	0.43^{a}	0.30^{bc}	0.03	0.04	0.78	0.12	<0.01

^{a,b,c}Means within the same row with the same letter are not different ($P > 0.05$).

[1]Treatment means are reported as least squares means. Five pigs (32.6 ± 2.6 kg) each fed one of five diets at 3 times maintenance in subsequent 14-d periods for 5 observations per diet. PCON = positive control; NCON = negative control; XYL = xylanase; PHY = phytase.

[2]The *P*-values for XYL, PHY, and XYL x PHY are among the 4 diets with reduced nutrient specifications

.digestibility and digestible content of Ca, but their combined effect was similar to their individual effect.

4.4.4 Digesta Passage Rate

The digesta passage rate to the ileum was similar between the positive and negative control diets (Table 4-5). Neither xylanase nor phytase affected the digesta passage rate.

4.5 Discussion

In the present study, feeding the diet with reduced nutrient specifications to grower pigs indeed reduced the measured digestible nutrient profile. Xylanase and phytase were supplemented to the negative control diet to improve nutrient digestibility and digestible nutrient content. Xylanase and phytase interacted to increase nutrient digestibility so that the effect of their combined supplementation was less than the sum of their individual effects.

4.5.1 Diet with Reduced Nutrient Specifications

In the present study, the diet with reduced nutrient specifications without enzymes had lower digestibilities of energy, DM, Ca, P, and a lower DE content compared to the positive control or the negative control plus enzymes. Diets with a reduced specification were used to mimic practical application of enzymes to enhance digestible nutrient profile and reduce the negative effects of NSP and phytate of the used, cost-effective

Table 4-5 Effect of xylanase and phytase supplementation on digesta passage rate and mean retention time of a diet with a reduced nutrient specifications and a positive control diet fed to grower pigs[1]

		Reduced nutrient specifications				Pooled	*P*-value[2]			
Passage rate to ileum	PCON	NCON	XYL	PHY	XYL + PHY	SEM	PCON vs. NCON	XYL	PHY	XYL x PHY
Passage rate, % Cr_2O_3/h	2.2	2.1	2.0	2.5	1.9	0.24	0.77	0.18	0.57	0.37
Mean retention time	5.3	5.4	5.3	5.3	5.5	0.12	0.51	0.95	0.54	0.16
Chromium appearance, h	2.1	2.0	2.1	2.1	1.8	0.23	0.75	0.90	0.65	0.51

[1]Treatment means are reported as least-square means. Five pigs (32.6 ± 2.6 kg) each fed one of five diets at 3 x maintenance in subsequent 14-d periods for 5 observations per diet. PCON = positive control; NCON = negative control; XYL = xylanase; PHY = phytase.

[2]The *P*-values for XYL, PHY, and XYL x PHY are among the 4 diets with reduced nutrient specifications

wheat by-product, so that improvements in digestible nutrient profile would be of nutritional and potential economic importance. To achieve the required reductions in digestible nutrient content, more wheat and less soybean meal was included in the negative control diet. As a result, the negative control diet had higher fibre content than the positive control diet. The majority of the fibre of the diets was provided by wheat and wheat millrun, and was thus arabinoxylans and cellulose (Basic and Stone, 1991) that can reduce nutrient digestibility and digestible nutrient content (Souffrant, 2001; Barrera et al., 2004). An increased NSP content in diets for pigs coincides with a reduction in the digestibility of nutrients (Fairbairn et al., 1999; Zijlstra et al., 1999; Nortey et al., 2007). Arabinoxylans encapsulate nutrients and prevent their digestion in monogastric animals such as swine that do not possess the enzyme xylanase that is required to digest arabinoxylans (Barrera et al., 2004). Generally wheat by-products contain more NSP than their parent grain (Slominski et al., 2004). Wheat millrun contains more bran and cell wall material (Dale, 1996) than the parent wheat and since arabinoxylans are found in the cell walls (Bedford, 1995) the encapsulating effect of diets based on millrun will be high. Therefore in diets based on millrun and formulated to be deficient in important nutrients, as was the case in the present study, the NSP are expected to have an important high negative effect on nutrient digestibility.

4.5.2 Xylanase and Phytase Supplementation

Xylanase and phytase interacted to improve ileal and total tract digestibility of energy and DM, and the DE and digestible nutrient content of the diets with reduced nutrient specification. An interaction between the two enzymes indicates that the combined

supplementation of the enzymes will provide an effect that is not equal to the sum of the effects of the individual enzymes. The interaction observed on the present study may be due to the individual enzymes releasing similar nutrients, and thus providing an overlapping response, as opposed to each releasing unique nutrients. In an earlier study (Nortey et al., 2007), supplementing xylanase and phytase individually to diets based on 20% wheat millrun improved energy, P, and AA digestibility, and the DE content. In such a situation, using both enzymes in combination in a diet will likely produce an effect which is not equal to the sum of the two individual enzymes due to overlapping effects of the two individual enzymes on a common response variable. This is likely what happened in this study. An interaction is possible in this study because supplemental xylanase is able to disrupt the cell wall matrix and expose encapsulated phytate and nutrients to phytase (Oryschak et al., 2002). Since this matrix is otherwise impermeable, once it is hydrolyzed, phytase enzymes can further hydrolyze phytate to release P and phytate bound nutrients for utilization by the pig. Nutrients that are capable of being bound by phytate include Ca, AA, and free starch (Selle et al., 2000); thus enzymatic hydrolysis of phytate can result in improvements on Ca, energy, and AA digestibility, as was the situation in this trial. However the response that was observed was not numerically equal to the sum of the two individual enzymes likely due, as explained previously, to a cross over effects of the two individual enzymes alone on a single response variable.

Independently, xylanase supplementation improved energy and DM digestibility and the improvements in energy digestibility and DE content with xylanase supplementation are consistent with previous research from our laboratory (Nortey et

al., 2007) and other researchers (Graham et al., 1998; Yin et al., 2000). The suggested mode of action of xylanase in improving nutrient digestibility is through a disruption or solubilization of the cell wall polysaccharides, resulting in a reduction or elimination of the encapsulating effects of arabinoxylans. The resulting increase in energy digestibility in the small intestine will consequently shift the site of and mode of digestion from microbial fermentation in the large intestine to enzymatic digestion in the small intestine (Dierick and Decuypere, 1994). Such a shift in site of digestion is more efficient and useful for the animal and will result in a greater net energy value of the feed. Removal of the encapsulating effects of fibre can thereby improve overall efficiency of utilization of dietary energy.

Xylanase did not affect AA digestibility and digestible contents in the present study. This is in contrast to previous studies using wheat based diets (Barrera et al., 2004; Nortey et al., 2007). Arabinoxylans entrap AA within their matrix, and supplementing xylanase may release the protein from the plant cell wall, thereby making the protein available for digestion and absorption as AA. In this trial however, although xylanase improved energy digestibility of the negative control diets, and indeed small numerical improvements in AA digestibility and also digestible AA contents were observed, there was not a corresponding increase in AA digestibility and content.

The role of phytase in AA digestibility is not understood, and different perspectives exist (Adeola and Sands, 2003). This has arisen because of conflicting results in the literature on the effects of phytase on amino acid and protein digestion. While some studies show an improvement in amino acid digestibility and retention with phytase addition (Mroz et al., 1994; Liao et al., 2005; Nortey et al., 2007) others have failed to

demonstrate an improvement (Bruce and Sundstol, 1995; Traylor et al., 2001). Generally it is suggested that for this phenomena to be understood well, it is important to identify and quantify all the factors involved in amino acid and protein response to phytase (Adeola and Sands, 2003). These authors group the main factors into three categories, 1) feed factors; 2) experimental protocol; and 3) animal factors. Feed factors include among others the quantity and source of phytin, and the quality of protein in the feed. Experimental protocol factors include processing of the diet and site of sampling (cannulated vs slaughter methods), while animal factors include species, genetics, and sex as these relate to GIT transit time, pH, and regulation of phytase activity in the brush border membranes.

In the present study, supplementation with xylanase and phytase individually produced an improvement in the total tract P digestibility, without interaction. A large proportion of the P in plant material is in the form of phytate (Ravindran et al., 1994; Liao et al., 2005). Pigs do not have the endogenous enzyme phytase that is needed to hydrolyze phytate and release P (Golovan et al., 2001). In addition to binding P and making it unavailable to the pigs, phytate can bind and form complexes with multivalent cations such as Ca, Zn, Fe, free starch, and AA thereby making them also unavailable (Selle et al., 2000). Supplementing phytase to the diet for pigs is thus one approach to hydrolyze the phytate molecule and improve P availability (Maga, 1982).

The improvement in P digestibility with xylanase supplementation is consistent with the result of an earlier trial (Nortey et al., 2007). The peripheral endosperm layers of wheat are important storage sites of phytate and P (Maga, 1982). Thus, a considerable amount of P will be encapsulated in the fibre matrix and be unreachable for digestion,

because pigs lack endogenous xylanase. Supplementing xylanase to the diets high in cereal fibre will therefore improve P digestibility indirectly, via enhanced interaction between P storage sites and enzymes in the gastro-intestinal tract. For P digestibility, xylanase and phytase did not interact, indicating that the cumulative individual effects of xylanase and phytase were equal to the effect of combined supplementation of xylanase and phytase in diets based on wheat and wheat millrun.

Content of ileal and total tract digestible P, and Ca, followed a similar pattern as digestibility. As expected, the negative control diet contained less digestible nutrients than the positive control diet. The inclusion of a positive control diet in the study illustrated that the supplementation of either xylanase or phytase improved the digestible nutrient content of the negative control diet, but did not reach a digestible nutrient content equal to the positive control. The digestible content of a nutrient in feed is a reflection of its dietary content and digestibility. Since the calculated and analyzed P and Ca content were equal, any improvement in digestibility caused by enzyme supplementation was reflected directly in their digestible nutrient contents.

Xylanase and phytase did not affect digesta passage rate in the present study. Digesta passage rate was measured to attempt to explain the interaction between xylanase and phytase supplementation observed in previous research of our laboratory (Oryschak et al., 2002), because increased passage rate can reduce nutrient digestibility. Digesta passage is related to the physical characteristics of the feed, such as the particle size, water holding capacity, bulkiness, and the size of the pig (Stanogias and Pearce, 1985). Dietary fibre content plays a role in determining digesta passage rate as well. Purified guar gum and cellulose reduced digesta passage rate in grower pigs (Owusu-

Asiedu et al., 2006). Feedstuff-bound soluble NSP in sugar beet pulp reduced digesta passage rate in pigs (Knudsen and Hansen, 1991). In contrast, bran and oatmeal by-products, representing insoluble and soluble NSP, respectively, increased the digesta passage rate in pigs (Latymer et al., 1985), but not NSP from soluble sources such as guar gum and pectin, indicating that the effect of solubility of NSP on transit time is inconsistent. In the present study, xylanase increased digestibility of nutrients likely through the methods described previously, i.e., through a rupture of the fibrous cell walls and releasing entrapped nutrients for the pig's endogenous enzymes. Simultaneously, xylanase may have increased the solubility of the intestinal contents likely through enzymatic hydrolysis of the plant NSP. Presumably, these effects were not large enough to cause significant changes in the digesta passage rate. Small differences in dietary fibre content do not change digesta passage rate (Cole et al., 1967a, b), and likely neither do small changes in soluble NSP in the gastro-intestinal tract caused by supplemental xylanase.

Xylanase and phytase were used individually or in combination in diets with a reduced nutrient specification. In conclusion, xylanase and phytase can improve digestibility of reduced nutrient specific diets based on millrun without influencing passage rate and retention time of nutrients.

5. EFFECT OF INDIVIDUAL OR COMBINED XYLANASE AND PHYTASE SUPPLEMENTATION ON SITE OF NUTRIENT DIGESTION OF A DIET WITH REDUCED NUTRIENT SPECIFICATIONS CONTAINING WHEAT AND MILLRUN RELATIVE TO A POSITIVE CONTROL DIET FED TO WEANED PIGS

5.1 Abstract

Millrun can effectively partially substitute wheat and be included in diets with a reduced nutrient specification for weaned pigs if enough uplift in nutrient availability can be provided by xylanase and phytase. The effects of xylanase (4375 U/kg of feed) and phytase (500 FTU/kg of feed) supplementation on energy digestibility, site of nutrient digestibility, and pH content in the gastrointestinal tract and on growth performance were studied in diets with reduced nutrient specifications fed to weaned pigs. The experiment was designed as a 2 x 2 factorial arrangement together with a positive control diet formulated to contain 3.50 Mcal DE/kg, 3.25g true digestible Lys/Mcal DE, 0.65% total P, and 0.80% Ca. The content of Lys, P, and Ca was reduced by 10% and the DE content by 150 kcal/kg in the other 4 diets, including the un-supplemented negative control diet. Weaned pigs (8.6 ± 0.5 kg) were housed individually and had free access to 1 of 5 diets for 21 d. Feces were collected on d 19. On d 20 and 21, pigs were euthanized and contents from segments of the

gastrointestinal tract were collected. Feeding a nutrient reduced diet reduced ($P < 0.01$) total tract energy digestibility. Xylanase and phytase interacted to improve ($P < 0.05$) the total tract DE content of the negative control diet by 240 kcal/kg. Xylanase improved ($P < 0.01$) energy digestibility of the NC in the mid jejunum and over the total tract by 63.0 and 4.6% respectively, and uplifted ($P < 0.01$) the DE content over the total tract by 240 kcal/kg. Phytase addition improved ($P < 0.05$) the DE content of the negative control diet by 160 kcal/kg. Feeding a nutrient reduced diet tended to reduce ($P = 0.074$) the pH content of the upper small intestine, and phytase raised ($P < 0.01$) the pH content of the upper mid small intestine. Xylanase improved ($P < 0.05$) BW, ADG, and G:F of the negative control diet at d 21 by 1.7 kg, 0.18 kg/d, and 0.06 respectively. On d 21, phytase improved BW ($P < 0.01$) and ADG ($P < 0.05$) of the negative control diet by 11.0 and 31.0%, respectively. Xylanase and phytase improved total tract DE content and performance of weaned pigs fed nutrient reduced diets. Phytase inclusion led to a more rapid return to alkaline conditions in the upper part of the small intestine. Exogenous enzymes can be used to improve digestibility of reduced-nutrient specific diets based on wheat and millrun for weaned pigs.

5.2 Introduction

The use of high fibre feedstuffs in swine diets may increase hindgut fermentation that results in inefficient energy utilization (Noblet et al., 1994). Pigs do not digest high fibre diets well, because the necessary endogenous enzymes for non-starch polysaccharides (NSP) hydrolysis are lacking (Barrera et al., 2004). Ingredients of plant origin also present P to pigs in the form of phytate P (Garcia-Estepa et al., 1999) that is

not easily available, because pigs not have endogenous phytase required to hydrolyze phytate P (Pointillart et al., 1984). Exogenous xylanase and phytase may improve digestibility of high fibre and phytate-based diets for swine (Graham et al., 1986; Li et al., 1996; Van der Meulen et al., 2001) by increasing the availability of energy, P, AA, and Ca (Nortey et al., 2007). Effects of xylanase and phytase on growth performance have been inconsistent (Bedford et al., 1992; Van Lunen and Schulze, 1996; Bedford and Schulze, 1998; Nortey et al., 2007). The kinetics of nutrient digestion within the gastrointestinal tract (GIT) may be studied using the slaughter technique and provide evidence for the changes in site of nutrient digestion and physiological characteristics of digesta following enzyme supplementation. The pH content of the GIT may be partly related to the VFA content that may be a result of the type and quantity of fibre in the diet, the buffering capacity of dietary nutrients and on the pKa of the GIT (Pluske et al., 1998; Pluske, 2003).

The hypothesis of the present study is that adding xylanase and phytase to a diet for weaned pigs based on wheat and wheat millrun and reduced in nutrient specification will improve energy digestibility and change digesta pH profile throughout the GIT. The objectives were: 1) to study the effect of xylanase and phytase and their interaction on the site of energy digestion in weaned pigs fed diets based on millrun, and 2) to study the effect of xylanase and phytase on the pH of the GIT.

5.3 Materials and Methods

5.3.1 Experimental Design and Diets

Effects of xylanase supplementation (0 or 4,375 units/kg feed) and (or) phytase (0 or 500 phytase units/kg feed) were studied in a 2 x 2 factorial arrangement in a diet with

reduced nutrient specifications (negative control), together with a positive control diet, for a total of 5 diets in a fractional factorial arrangement. The positive control diet was formulated to be at requirement for all nutrients. The negative control diet was formulated to be low in energy by 150 kcal/kg, and in dietary Lys, Met, Thr, P, and Ca by 10% (Table 5.1). The xylanase was endo-1, 4-β-xylanase (EC 3.2.1.8; Porzyme 9300; Danisco Animal Nutrition, Marlborough, UK), and the phytase was 6-phytase (EC 3.3.26; Phyzyme XP; Danisco Animal Nutrition). All diets contained 7.5% wheat millrun that had been steam-pelleted (Dawn Foods, Saskatoon, Saskatchewan, Canada) to reduce bulk density and facilitate transport and was reground on a hammer mill across a 4-mm screen (New-Life Feeds, Saskatoon, Saskatchewan, Canada). The millrun contained the screenings, bran, and short fractions but not the middlings fraction after flour milling of hard red spring wheat. The wheat control diet was formulated to contain 3.50 Mcal/kg DE and 3.25 g true ileal digestible lysine/Mcal DE. To the diets, 1% acid-insoluble ash was added to serve as marker for digestibility.

5.3.2 Experimental Procedures

The animal protocol for the study was approved by the University of Alberta Faculty Animal Policy and Welfare Committee, and followed established principles (CCAC, 1993). The experiment was conducted at the Swine Research and Technology Centre of the University of Alberta (Edmonton, Alberta, Canada)

Table 5-1 Ingredient and nutrient composition (as-fed basis) of the negative and positive control diets

Item	Positive control	Negative control[1]
Ingredients, %		
Wheat	55.46	64.93
Soybean meal	26.00	21.00
Wheat millrun	7.50	7.50
Canola oil	3.40	0.40
Spray dried whey	1.50	1.00
Herring meal	1.50	1.00
Limestone	1.22	1.23
Celite	1.00	1.00
Dicalcium phosphate	0.73	0.36
Vitamin premix[2]	0.50	0.50
Mineral premix[3]	0.50	0.50
L-Lys HCl	0.30	0.35
Salt	0.20	0.20
Choline chloride (60%)	0.12	–
L-Thr	0.06	0.03
DL-Met	0.01	–
Calculated nutrient content[4]		
DE, Mcal/kg	3.50	3.35
ME, Mcal/kg	3.27	3.12
CP, %	22.40	20.96
CF,%	2.69	2.76
True digestible Lys, g/Mcal DE[5]	3.25	3.16
Total Lys, %	1.35	1.25
True ileal digestible Lys, %	1.14	0.81
Total P, %	0.65	0.55
Available P, %	0.36	0.27
Ca, %	0.80	0.70

[1]Xylanase was included at 167 g/1,000 kg of finished feed, and phytase was included at 100 g/1,000 kg of finished feed to create the enzyme-supplemented diets.

[2]Provided the following per kilogram of diet: vitamin A, 8,250 IU; vitamin D_3, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine, 1 mg; D-biotin; 0.2 mg; and vitamin B_{12}, 0.025 mg.

[3]Provided the following per kilogram of diet: Zn, 100 mg as $ZnSO_4$; Fe, 80 mg as $FeSO_4$; Cu, 50 mg as $CuSO_4$; Mn, 25 mg as $MnSO_4$; I, 0.5 mg as $Ca(IO_3)_2$; and Se, 0.1 mg as Na_2SeO_3.

[4]Calculated content: positive control diet, 0.30% digestible Met, 0.66% digestible Thr, 0.30% digestible Cys, and 0.20% digestible Trp; negative control diet, 0.27% digestible Met, 0.57% digestible Thr, 0.29% digestible Cys, and 0.19% digestible Trp.

[5]Calculated content: positive control diet: 3.25 and negative control diet; 3.16 g true digestible Lys/Mcal of DE (0.91 and 0.81% apparent digestible Lys respectively) and an ideal pattern of apparent digestible AA compared to Lys [i.e., Thr, 60; and Met, 30 (NRC, 1998)].

Twenty five weaned pigs (Large white x Duroc; Genex Hybrid; Hypor, Regina, Saskatchewan, Canada; initial BW, 8.6 ± 0.5 kg) were housed individually in pens each measuring 1 x 0.5 x 0.8 m (length x width x height). The dimensions of the pens allowed freedom of movement of the pigs during the entire experiment. The floor of the pens was plastic coated expanded metal and each crate had an individual feeder and nipple drinker. Each pig was randomly fed 1 of 5 diets in 1 period of 21 d resulting in 5 observations per diet. Pigs were weighed at the beginning of the experimental period (d 0), and weekly thereafter (d 7, 14, and 21). On each weigh day, feed disappearance was determined, and the combined data were used to calculate ADG, ADFI, and G:F.

Feces were collected for 8 h on d 18 and 19, pooled by pig and frozen at –20ºC. On d 20 and 21, pigs were euthanized and the GIT removed. The GIT was laid on a table so that the small intestine was divided into 4 segment of equal length. The contents of the stomach, upper small intestine (segment 1), mid jejunum (segment 2), lower-mid small intestine (segment 3), lower small intestine (segment 4), cecum, mid-colon, and rectum were carefully emptied into plastic containers. The pH of segments was measured (Accumet Basic AB 15 pH meter, Fisher Scientific) and samples were immediately frozen at –20ºC for later analyses.

5.3.3 Chemical Analyses

Prior to analyses, feces and digesta were thawed, homogenized, and freeze-dried. Feed and freeze-dried feces were ground finely over a 1-mm screen. Digesta was ground over a 0.5-mm screen and together with feed was analyzed for NSP. Feed and feces were analyzed for DM by drying at 135ºC in an airflow-type oven for 2 h (method 930.15; AOAC, 1990). Acid-insoluble ash content was analyzed by the method of Van Keulen and Young (1977). The GE of feed, feces, and digesta was analyzed adiabatically using an automatic bomb calorimeter (model AC-300, Leco Corporation, St. Joseph, MI). Diet and lower small intestinal samples were analyzed for soluble and insoluble NSP and constituent sugars by GLC (Englyst and Hudson, 1987).

Based on the results of chemical analysis, ileal and total-tract digestibility of GE and DM, and DE content were calculated using the acid-insoluble ash concentration of feed, digesta and feces (Adeola, 2001). The energy and AIA contents of 3 additional segments of the small intestine (upper, upper-mid, and lower-mid small intestine) were analyzed to determine energy digestibility, and together with results from the terminal ileum, energy disappearance along the GIT was determined.

5.3.4 Statistical Analyses

To compare the differences in total tract digestibility of DM and energy between the diets, data was analyzed by ANOVA using the GLM procedure within SAS (SAS Inst. Inc., Cary, NC). Pig was considered the experimental unit. Ileal digestibility data along the different segments of the GIT and also the performance data were analyzed by the PROC MIXED procedure within SAS as a Completely Randomized Design. Main

effects of xylanase, phytase, and their interaction term were determined in the diets with reduced nutrient specifications. The negative control diet was compared to the positive control diets using a contrast statement. Treatment means were separated using the probability of difference solely for variables for which an interaction between the main effects was detected ($P < 0.10$). Differences were considered significant if $P < 0.05$ and were described as tendencies if $0.05 < P < 0.10$.

5.4 Results

5.4.1 Energy and DM Digestibility

Total tract energy digestibility of the positive control diet (85.5%: Table 5.2) was depressed ($P < 0.05$) by 3.6 %-units in the negative control diet to 81.9%. In the mid jejunum and colon, the negative control diet tended to have ($P = 0.072$ and 0.066, respectively) a reduced energy digestibility compared to the positive control diet. Xylanase and phytase tended to interact on energy digestibility in the mid colon ($P < 0.10$) and for the total tract ($P < 0.10$). Xylanase improved ($P < 0.01$) energy digestibility in the mid jejunum and for the total tract. Xylanase improved ($P < 0.01$)

Table 5-2. Effect of xylanase and phytase supplementation on apparent ileal and total tract energy and DM digestibility and digestible energy content in different segments of the GIT of a diet with reduced nutrient specifications and a positive control diet fed to weaned pigs[1]

Item	PCON	Reduced nutrient specifications				Pooled SEM	*P*-value[2]			
		NCON	XYL	PHY	XYL + PHY		PCON vs. NCON	XYL	PHY	XYL x PHY
Energy digestibility, %										
Mid jejunum	49.6	36.5	59.5	43.6	51.2	4.87	0.072	0.005	0.901	0.132
Terminal ileum	60.2	55.2	62.6	59.6	59.2	8.35	0.687	0.687	0.953	0.656
Mid colon	84.3	81.2	84.3	83.7	82.4	1.09	0.066	0.422	0.855	0.059
Total tract	85.5[a]	81.9[b]	85.7[a]	84.0[ab]	84.9[a]	0.76	0.003	0.005	0.433	0.070
DE, Mcal/kg										
Mid jejunum, as-is	1.99	1.41	2.34	1.71	2.04	0.19	0.045	0.004	0.988	0.136
Terminal ileum, as-is	2.40	2.09	2.46	2.30	2.37	0.32	0.624	0.591	0.935	0.652
Mid colon, as-is	3.38	3.18	3.32	3.30	3.29	0.05	0.001	0.580	0.195	0.131
Total tract, DM	3.86[a]	3.58[c]	3.82[ab]	3.74[b]	3.82[ab]	0.03	<0.001	<0.001	0.021	0.045

[a,b]Means within the same row with the same letter are not different (*P > 0.05*).

[1]Treatment means are reported as least squares means. Twenty five weaned pigs (8.6 ± 0.5 kg) each fed one of five diets in one period of 21 d for 5 observations per diet. PCON = positive control; NCON = negative control; XYL = xylanase; PHY = phytase.

[2]The P-values for XYL, PHY, and XYL x PHY are among the 4 diets with reduced nutrient specifications.

energy digestibility of the negative control total tract diet by 3.6 %-units for the total tract and 23 %-units in the mid jejunum. Phytase did not affect energy digestibility.

The positive control had a higher DE content than the negative control diet in the mid jejunum ($P < 0.05$: Table 5.2), mid-colon ($P < 0.01$), and over the total tract ($P < 0.001$). Xylanase improved ($P < 0.01$) the DE content of the negative control diet in the mid jejunum by 0.93 Mcal/kg. Xylanase and phytase interacted to improve ($P < 0.05$) the total tract DE content of the negative control diet. Xylanase and phytase independently improved ($P < 0.05$) the total tract DE content of the negative control diet by 0.24 and 0.16 Mcal/kg, respectively, but the effect of the combined supplementation did not equal their cumulative effects. The DE content of the negative control diet with xylanase equaled the DE content of the positive control diet.

NSP Digestibility. The NSP results obtained for the present study did not turn out to be useful. Most of the sugars analyzed had negative digestibility coefficients. Therefore, the data is not discussed. However, for those sugars that showed a positive digestibility, a clear pattern was not observed and conclusion could not be drawn (Appendix 1).

5.4.2 Digesta pH

Pigs fed the negative control diet had a reduced ($P < 0.05$; Table 5.3) digesta pH in the upper mid small intestine. Xylanase and phytase interacted ($P < 0.05$) to increase the pH of the mid jejunum. Phytase alone increased ($P < 0.01$) the pH in the mid jejunum by 0.53 to pH 6.51. In the lower-mid small intestine, the two enzymes together tended to interact ($P = 0.08$) to affect digesta pH of the GIT. By the end of the small intestine, diet and enzymes did not affect digesta pH.

Table 5-3. Effect of xylanase and phytase supplementation on pH of contents of different portions of the small intestine in weaned pigs[1]

		Reduced nutrient specifications				Pooled	*P*-value[2]			
pH	PCON	NCON	XYL	PHY	XYL + PHY	SEM	PCON vs. NCON	XYL	PHY	XYL x PHY
Upper small intestine	5.84	5.82	5.94	5.90	5.90	0.074	0.420	0.779	0.436	0.836
Upper mid small intestine	6.34^{ab}	5.98^{c}	6.14^{bc}	6.51^{a}	6.22^{abc}	0.104	0.024	0.532	0.008	0.037
Lower mid small intestine	6.57^{ab}	5.97^{b}	6.33^{ab}	6.71^{a}	6.22^{ab}	0.230	0.081	0.781	0.187	0.084
Lower small intestine	6.26	5.99	6.11	6.31	6.22	0.221	0.398	0.947	0.640	0.398

[a,b,c]Means within the same row with the same letter are not different ($P > 0.05$).

[1]Treatment means are reported as least squares means. Twenty five weaned pigs (8.6 ± 0.5 kg) each fed one of five diets in one period of 21 d for 5 observations per diet. PCON = positive control; NCON = negative control; XYL = xylanase; PHY = phytase.

[2]The P-values for XYL, PHY, and XYL x PHY are among the 4 diets with reduced nutrient specifications.

5.4.3 Growth Performance

Pigs fed the negative control diet were 1.4 and 2.7 kg lighter ($P < 0.05$; Table 5.4) on d 14 and 21, respectively, than pigs fed the negative control diet. Xylanase improved ($P < 0.05$) BW of pigs fed the negative control diet on d 21 by 1.7 kg. On d 14, phytase tended to improve ($P = 0.07$) BW by 1.0 kg and improved ($P < 0.01$) BW on d 21 by 1.1 kg. Xylanase and phytase did not interact for BW.

Reducing the nutrient content of the diet did not affect ADFI. For d 15 to 21, xylanase and phytase tended to interact ($P = 0.08$) to improve the ADFI of pigs fed the negative control diet by 0.19 kg/d. Phytase tended to improve the ADFI between d 8 to 14 (P = 0.09) by 0.14 kg/d, and between d 15 to 21 ($P = 0.07$) by 0.23 kg/d. Overall from d 0 to 21, enzymes did not affect ADFI.

Reducing the energy content of the diet reduced ($P < 0.01$) ADG between d 8 to 14 and d 15 to 21 by 0.18 and 0.20 kg/d respectively, and tended to reduce ($P = 0.05$) overall ADG for d 0 to 21 by 0.14 kg/d. Xylanase improved ($P < 0.05$) ADG for d 15 to 21 by 0.16 kg/d. Phytase tended to improve ($P = 0.05$) ADG for d 8 to 14, improved ($P < 0.05$) ADG for d 15 to 21, and tended to improve ($P = 0.08$) overall ADG for d 0 to 21. Xylanase and phytase did not interact for ADG.

Reducing the nutrient content of the diets reduced G:F for d 8 to 14 ($P < 0.05$) by 0.11, for d 0 to 21 ($P < 0.01$) by 0.15, and tended to reduce G:F for d to 7 and 15 to 21 by 0.21 and 0.70 respectively. Xylanase improved ($P < 0.05$) overall G:F for d 0 to 21 by 0.09. Phytase did not affect G:F, and an interaction between xylanase and phytase was not observed.

Table 5-4. Effect of wheat millrun inclusion level, and xylanase and phytase supplementation, either individually or in combination to wheat-based diets on performance of weaned pigs over time[1]

Item	PCON	Reduced nutrient specifications				Pooled SEM	*P*-value[3]			
		NCON	XYL	PHY	XYL + PHY		PCON vs. NCON	XYL	PHY	XYL x PHY
BW, kg										
d 7	11.4	11.2	11.3	11.2	11.8	0.58	0.675	0.263	0.420	0.295
d 14	16.8	15.4	16.0	16.4	16.8	0.90	0.034	0.231	0.068	0.771
d 21	21.8	19.1	20.8	21.2	22.1	0.99	<0.001	0.019	<0.01	0.393
ADFI, kg/d										
d 0 to 7	0.45	0.57	0.50	0.55	0.52	0.06	0.161	0.364	0.952	0.684
d 8 to 14	1.05	0.97	1.00	1.11	1.05	0.06	0.304	0.813	0.099	0.450
d 15 to 21	1.09[ab]	1.00[b]	1.18[ab]	1.23[a]	1.19[ab]	0.06	0.299	0.260	0.066	0.083
d 0 to 21	0.86	0.84	0.89	0.96	0.92	0.08	0.847	0.993	0.344	0.569
ADG, kg/d										
d 0 to 7	0.39	0.37	0.37	0.36	0.45	0.05	0.680	0.324	0.453	0.352
d 8 to 14	0.77	0.59	0.68	0.74	0.71	0.04	<0.01	0.468	0.048	0.162
d 15 to 21	0.78	0.58	0.74	0.76	0.83	0.05	<0.01	0.026	0.014	0.353
d 0 to 21	0.65	0.51	0.59	0.62	0.66	0.05	0.049	0.191	0.082	0.651
G:F										
d 0 to 7	0.89	0.68	0.74	0.69	0.88	0.08	0.103	0.195	0.408	0.474

Table 5-4 (continued). Effect of wheat millrun inclusion level, and xylanase and phytase supplementation, either individually or in combination to wheat-based diets on performance of weaned pigs over time[1]

		Reduced nutrient specifications				Pooled	*P*-value			
Item	PCON	NCON	XYL	PHY	XYL + PHY	SEM	PCON vs. NCON	XYL	PHY	XYL x PHY
d 8 to 14	0.73[a]	0.62[b]	0.69[ab]	0.67[ab]	0.68[ab]	0.03	0.025	0.247	0.553	0.347
d 15 to 21	0.73	0.60	0.63	0.62	0.70	0.05	0.073	0.226	0.388	0.565
d 0 to 21	0.78	0.63	0.69	0.66	0.75	0.04	<0.01	0.047	0.210	0.579

[a,b]Means within the same row with the same letter are not different ($P > 0.05$).

[1]Treatment means are reported as least squares means. Twenty five weaned pigs (8.6 ± 0.5 kg) each fed one of five diets in one period of 21 d for 5 observations per diet. PCON = positive control; NCON = negative control; XYL = xylanase; PHY = phytase.

[2]The P-values for XYL, PHY, and XYL x PHY are among the 4 diets with reduced nutrient specifications

5.5 Discussion

In the present study, feeding diets with reduced nutrient specifications to weaned pigs reduced energy digestibility for individual segments of the GIT and for the total tract, reduced growth performance, and affected pH content of the segment of the GIT. Adding xylanase to nutrient reduced diets improved energy digestibility, DE content, BW, and G:F. Phytase increased the pH content, improved total tract DE content, and BW. The effects on pH content were more pronounced in the proximal part of the small intestine. Xylanase and phytase interacted to improve DE content of diets with reduced nutrient specifications: the improved DE content was not the sum of the individual enzyme effects.

5.5.1.Nutrient Reduction

The requirements for specific nutrients for pigs are dependent on stage of growth, genetic strain, gender, environment, and other factors (NRC, 1998), and have been developed using different models, and deviations from these models can cause a drop in performance. For weaned pigs, selection of feedstuffs for feed formulation is important to meet requirements and is based on 3 major criteria (Tokach et al., 2003): 1) adjusting the pigs to the simplest and relatively lowest cost diet as soon as possible post-weaning, 2) maximizing feed intake to ensure that the pig receives adequate nutrients without mobilizing body reserves and, and 3) formulating initial diets with highly digestible ingredients that will complement the pattern of digestive enzymes, and digestive enzyme development in the GIT.

For the present study, ingredient selection was not based on the above 3 criteria. Wheat millrun contains more of less-digestible ingredients such as fibre compared to wheat (Souffrant, 2001; Svihus and Gullord, 2002; Slominski et al., 2004). Feeding a diet that is high in fibre can be detrimental to young pigs, because pigs do not have the necessary enzymes and hence do not digest fibre well (Barrera et al., 2004). In the dietary formulations for the present study, energy was reduced by reducing the content of highly digestible feedstuffs such as soybean meal, herring meal, spray dried whey, and canola oil, while keeping the level of wheat millrun constant (Table 5.1). The NSP can act as anti-nutritional factors (ANF; Stanogias and Pearce, 1985) and compromise the digestibility of other nutrients (Nortey et al., 2007); therefore, energy digestibility is expected to be reduced in a diet with reduced nutrient specifications, as was the case in the present study.

A large proportion of dietary fibre to pigs is fermented in the hindgut (Noblet et al., 1996). The efficiency of utilization of ME to NE varies and depends on the purpose of energy utilization and also with dietary characteristics (Just, 1982). The conversion efficiency of ME derived from hindgut fermentation to NE is lower than energy derived from enzymatic digestion in the anterior GIT (Shi and Noblet, 1993; Noblet et al., 1996). In the present study, supplementing xylanase to the diet with reduced nutrient content improved digestibility in the upper jejunum.

The pH of the GIT contents was influenced by dietary nutrient level. Digesta pH may not be dependent solely on the quantity of VFA (Pluske, 2003), but also depends on the acid dissociation constant (pK_a), the proportion of specific VFA in the intestinal digesta, and the buffering capacity of dietary nutrients such as protein (Pluske et al.,

1998). The pK_a of an acid is a measure of ease and extent of ionization and is a method of measuring the relative strength of acids. The dietary fibre content for the present study were relatively constant, hence the changes in digesta pH may indicate buffering capacity of dietary nutrients rather than quantity of fibre or VFA playing a role.

A reduction in growth performance with reduced dietary nutrient content may be a direct result of reduced dietary nutrient content and (or) digestibility. For pigs to realize their full genetic potential in a given environment, required nutrients must be supplied in the right quantities and form (NRC, 1998). For the present study, the important nutrients energy, AA, P, and Ca were reduced by 10% in the negative control diet, resulting in a reduced dietary EE content and a minor increase in fibre content (Table 5.1). Pigs do not digest NSP well, because the porcine GIT does not produce the necessary enzymes (Barrera et al., 2004). Non-starch polysaccharides reduce digestibility of nutrients (Bell et al., 1983) including AA and DM. With reduced dietary supply of nutrients and a lowered nutrient digestibility, growth performance will reduce as observed in the present study, and thus provides an ideal platform to measure uplift in nutrient digestibility and growth performance following dietary manipulation via enzyme supplementation.

5.5.2 Xylanase Supplementation

Xylanase supplementation improved the digestibility and DE contents of the diet with reduced nutrient content. Within individual segments, the greatest impact of enzymes was observed with xylanase in the mid jejunum, and coincided with the improved total tract energy digestibility and DE contents. Research using similar slaughter techniques

has determined that generally the digestibility of NSP in the upper small intestine using oat bran (Bach Knudsen et al., 2006) and sorghum-acorn diets (Morales et al., 2002) is small. The latter also indicated that the disappearance of NSP in the upper small intestine is mainly due to microbial fermentation giving rise to volatile fatty acids. Results of the present trial indicate that xylanase supplementation caused hydrolysis of NSP to occur in the upper parts of the GIT, specifically the small intestine. The pig benefits energetically following such a shift, because enzymatic hydrolysis in the small intestine is a more efficient process of energy transformation than hindgut fermentation (Noblet et al., 1994). Further evidence is provided by Taverner and Campbell (1998) who demonstrated that including a carbohydrase, β-glucanase, in diets for grower pigs can lead to increased digestibility of energy and protein by altering the site of digestion from the large to the small intestine. For this trial the uplift provided by xylanase resulted in digestibility coefficients that were similar to that of the positive control diet. For the diets with a reduced nutrient content, limitations imposed on nutrient availability can be seen in 2 ways: first, digestible nutrient content was reduced, and second, fibre content was slightly increased. Supplementing xylanase to the diets complemented the pigs' lack of endogenous xylanase (Li et al., 1996) and improved the nutritional value of the feed. In the present study, xylanase supplementation improved energy digestibility and DE contents so that the improved coefficients were similar to the positive control diet, similar to previous studies (Graham et al., 1986; Nortey et al., 2007) indicating that adding fibre-degrading enzymes to diets high in NSP can improve energy digestibility and utilization.

Xylanase supplementation has had inconsistent effects on growth performance in swine (Bedford and Schulze, 1998). Xylanase supplementation improved ADG in pigs fed corn (Van Lunen and Schulze, 1996), rye (Bedford et al., 1992) and wheat-based diets (Zijlstra et al., 2004), but did not affect ADG, improved G:F, and reduced ADFI in pigs fed millrun-based diets (Nortey et al., 2007). For the present study, xylanase improved growth performance, consistent with improved nutrient digestibility. The improved performance in the present study is a result of removal of the negative effects of NSP present in the diets. Xylanase did not increase ADFI, but the observed increase in BW and ADG indicate that improved performance were due mainly to improved G:F. Xylanase did not affect pH of the GIT indicating that gastrointestinal modifications by xylanase did not change the pK*a* and VFA concentrations that may cause drastic changes in intestinal pH (Pluske et al., 1998).

5.5.3 Phytase Supplementation

Most feedstuffs for swine are of plant origin. In plants, phytic acid is the major storage form of P (Garcia-Estapa et al., 1999). Most of the phytic acid in wheat is located in the bran; in wheat containing about 0.32% phytic acid, 87% is contained in the aleurone layer, 13% in the germ, and 2% in the endosperm (O'Dell et al., 1972). For the type of diet used in the present study containing the by-product millrun, the content of phytic acid will be high compared to a diet without by-product. Pigs do not produce phytase that is necessary for P digestion (Pointillart et al., 1984) and the presence of phytic acid in the diet not only interferes with P availability, but also may reduce the availability of other nutrients such as protein, starch, lipids, and other minerals (Thompson and Yoon,

1984; Nortey et al., 2007). Complementing the pig's lack of phytase production by supplementation of exogenous phytase can therefore not only improve P digestibility, but also improve digestibility of other nutrients. Adding phytase to the negative control diets in the present study improved total tract DE content. Phytase increased the pH content of the mid jejunum indicating a more rapid shift of the GIT contents from a state of acidity in the stomach to one of neutrality. In addition to producing enzymes for carbohydrate digestion, the small intestine also secretes alkaline pancreatic juice and bile fluids that convert the acid chyme entering from the stomach to a more alkaline material (Yen, 2001). The conversion to more alkaline conditions provides an optimum pH environment for intestinal enzyme activity. Providing an optimum environment for enzymatic action in the proximal small intestine may reduce the amount of nutrients that will be fermented in the large intestine, thereby improving nutrient utilization. Results of the present study indicate that phytase may help facilitate that shift. Phytase inclusion improved BW at d 21, which could be partly due to improved energy utilization.

5.5.4 Xylanase and Phytase Interaction

Xylanase and phytase interacted for total tract energy digestibility, and tended to interact for energy digestibility in the mid-colon and total tract. The effects of xylanase and phytase on nutrient digestibility were not synergistic. For synergy to occur, the combined supplementation of xylanase and phytase must provide an effect that is at least equal to the sum of effects of the individual enzymes. The lack of synergy may indicate that the effects of the individual enzymes may overlap in the response. In plants

phytic acid is the primary P reserve in cereal grains and legumes and accounts for up to 85% of total P (Oatway et al., 2001). It has 12 replaceable protons and this allows it to form complexes with multivalent cations and positively charged proteins. As a result of this, phytic acid can exist in many forms; phytate being the calcium salt, and phytin being the calcium/magnesium salt of phytic acid. Phytic acid is hydrolyzed to release free P during germination by endogenous phytase which is found in the outer aleurone layers of the seed. In addition to minerals and proteins, phytic acid can also bind to starch, and in so doing can alter the solubility, functionality and digestion of these food ingredients. Adding exogenous phytase to a diet that contains indigenous phytase can, in addition to releasing P, release other bound nutrients like proteins and starch thereby making them available for enzymatic hydrolysis. In wheat, NSP include cellulose, pectins, β-glucans and arabinoxylans (Souffrant, 2001). Arabinoxylans and cellulose are the most important of these ANF (Zijlstra et al., 1999) and are found in the outer cell walls, similar to phytate. NSP entrap nutrients and since pigs do not have the endogenous xylanase enzymes needed to digest arabinoxylans (Barrera et al., 2004) their presence can render other macronutrients unavailable (Bell et al., 1983). Thus combined supplementation of xylanase and phytase in a diet may not produce a synergistic effect due to an overlapping effect of the individual enzymes on common response variable. Xylanase and phytase may complement each other, because xylanase breaks down the cell wall matrix and hydrolyses otherwise unavailable nutrients, while simultaneously allowing phytase to hydrolyze phytate and release P and other nutrients (Oryschak et al., 2002).

In conclusion, phytase inclusion led to an increase in the pH content of the proximal small intestine which may reduce the amount of nutrients that may be fermented in the large intestine. This shift may ultimately result in improvement in energy utilization and therefore improved performance. Xylanase and phytase have the potential to improve nutrient digestibility of a diet with reduced nutrient specifications to coefficients similar to a positive control. The uplift in digestibility can improve growth performance, which in the present study was a result of improved G:F.

6. GENERAL DISCUSSION

In commercial swine operations, feed represents around 50% of the total cost of production and 75% of the variable cost (Patience et al., 1995). Energy from feed is the single largest contributor to feed costs (de Lange and Birkett, 2004). For selection of feedstuffs for swine diets, several factors have to be taken into account. First, the nutrient composition of the ingredient, cost, availability, and palatability need to be characterized and understood (Patience et al., 1995). Second, the pig's nutrient requirements should be reached with the particular ingredient included in the complete feed. Especially for by-products, the digestible nutrient profile is not well characterized.

Wheat is a major crop in Western Canada. For 2007, the Canadian Wheat Board forecasted a wheat production of 20.7 million tonnes. Wheat millrun is a by-product of dry milling wheat into flour for human consumption and is abundant in Western Canada. The inclusion of wheat by-products such as millrun in swine diets might be economically attractive but also poses challenges. Wheat by-products may contain higher amounts of fibre, proteins and minerals than wheat (Slominski et al., 2004); however, these nutrients are normally not readily available to the pig. The high fibre content in by-products hinders access to nutrients enclosed within the fibre matrix to digestive enzymes and, coupled with pigs' lack of endogenous xylanase production, may cause these nutrients to be less digestible to the pig. The presence of phytate, the

main form of P in plants also reduced digestibility of P, AA, starch, and minerals (Selle et al., 2000).

For wheat millrun to be effectively included in swine diets the digestible nutrient profile needs to be properly characterized and limitations in nutrient digestibility should be identified. A series of experiments were conducted as part of the present thesis to fill some of the gaps in knowledge for the feeding of wheat by-products to swine.

6.1 Millrun Inclusion

In Exp. 1, the inclusion of millrun in swine diets reduced nutrient digestibility and DE content. Calculated dietary DE content was higher than analyzed content, indicating that the DE content of millrun used for the study was overestimated. Millrun may contain bran, shorts, middlings, flour, and offal from the tail of the mill. For Exp. 1, the millrun that was used did not contain the middlings fraction, which has more starch and contains more energy than other fractions such as bran. At the time of the trial, the exclusion of middlings was not known, so the DE content of the millrun was an estimate that was based on the assumed by-product stream, illustrating the problems of variability in nutritional quality among batches of by-products. Limitations in knowledge of the digestible nutrient content of feedstuffs may produce unpredictable growth performance. With a higher fibre content of the millrun-based diets and resultant increase in the ANF effect (Stanogias and Pearce, 1985), nutrient digestibility was reduced (Barrera et al., 2004). Millrun inclusion also reduced ADG, G:F, and BW, but not ADFI. Normally it is expected that, within limits, pigs can compensate for a low density DE diet by increasing their feed intake. However, in this Exp., the expected

increase in ADFI was lacking, likely due to the presence of NSP that may have prevented the expected compensatory increase in ADFI (NRC, 1998).

In Exp. 2, individual by-products that make up millrun were incorporated into a basal diet and fed to growing pigs. Adding by-products depressed digestibility and digestible nutrient content in comparison to a wheat basal diet. The extent of the reduction in digestibility depended on the specific by-product. The reductions in digestibility that occurred following millrun addition were large and not typical in comparison to the rest of the diets. The used millrun had been steam pelleted prior to being used in feed that may have inactivated endogenous enzymes such as xylanase (Cowieson et al., 2005). Differences in digestibility among the individual co-products indicate that the nutritional value of wheat millrun depends to a very large extent on the proportions of the individual co-products that make up millrun. Thus, if millrun contains more high value middlings and less low value bran, its nutritional value will increase accordingly. Results of Exp. 2 thus validated those of Exp. 1, suggesting that the absence of the starchy middlings stream in the millrun reduced DE content and GE digestibility compared to expectation.

In Exp. 3 and 4, cannulated grower pigs and weaned pigs, respectively, were fed positive and negative control diets based on millrun. Digestibility of nutrients including energy, AA, P, Ca, and DE content for Exp. 3, and energy and growth performance for Exp. 4 were reduced in diets with a reduced nutrient content. In Exp. 4, feeding a diet with reduced nutrient content reduced the pH in the upper-mid small intestine. This reduction may adversely affect nutrient digestibility, because intestinal enzymes need an alkaline environment for optimum performance (Yen, 2001).

6.2 Xylanase and Phytase Supplementation

In Exp. 1, addition of xylanase to diets based on millrun increased energy, AA, and P digestibility and the DE content. In addition, xylanase improved G:F and depressed ADFI. Phytase addition improved digestibility of P, DM, AA, and the DE content. Performance variables did not improve following enzyme supplementation. The lack of a corresponding performance improvement may be due to a lack of knowledge at that time of the nutrient profile of millrun, leading to an error in feed formulation. An ensuing nutrient imbalance may then have arisen causing a lack of response. Between the two enzymes, a synergy existed in P digestibility, because the effect of the combined supplementation of the 2 enzymes was greater than the sum of effects of supplementation of single enzymes.

In Exp. 2, the co-product streams responded differently to xylanase supplementation, indicating that the substrate present in each by-product was different. The DE content of millrun as calculated by the method of difference was 2.41 kcal/kg DM, lower than was initially assumed (2.90 kcal/kg DM) for feed formulation in Exp. 1, and confirmed the initial suspicion. With certainty, the digestible content of AA, P, and Ca was measured for millrun and other co-products.

In Exp. 3, the impact of supplemental enzymes on rate of digesta passage through the GIT was tested. A faster rate of digesta passage through the GIT will provide less contact time with endogenous enzymes, and nutrient digestibility may decline. Physical and chemical characteristics of feed such as soluble versus insoluble fibre affect digesta passage rate (Latymer et al., 1985; Knudsen and Hansen, 1991; Owusu-Asiedu et al.,

2006). Results from Exp. 3 indicate that the improved nutrient digestibility and digestible nutrient content following enzyme addition were due to a direct effect of enzyme on substrate and not to an indirect effect on passage rate. Xylanase and phytase in combination improved nutrient digestibility but their effect was not synergistic.

As a high fibre ingredient, part of millrun is digested in the swine hindgut (Noblet et al., 1996). Utilization of energy digested in the foregut is more efficient than energy fermented in the hindgut, because the former converts ME into NE more efficiently (Shi and Noblet, 1993; Noblet et al., 1996). In Exp. 4, energy digestibility in the upper small intestine was improved with xylanase addition. The pH content of the upper-mid small intestine was also higher and more conducive to endogenous enzymes with phytase addition. Combined, the effects lead to improved nutrient digestibility and explain the improved growth performance that was observed in this and previous studies.

6.3 Limitations to the Studies

For Exp. 1, our lack of knowledge of the nutrient profile of millrun resulted in an overestimated nutrient profile of millrun. Commercially available millrun might be pelleted by the flour mills to facilitate ease of transportation, but pelleting will change the physical and chemical characteristics. Pelleted millrun might thus lead to results not typical of other by-products of wheat dry-milling. Due to its high fibre content, high dietary inclusion of millrun may cause difficulties at digesta sampling by blocking the barrels of the cannulas. Frequent sampling and constant removal of compacted digesta from the cannula barrel on sampling days will reduce these problems. Another limitation was the indirect approach to determine the rate of energy uptake along the

GIT. Initially, portal vein catheterization was proposed to study nutrient absorption kinetics; however, surgeries could not be performed at the planned time of study due to unexpected circumstances. The resulting approach still provided useful data.

The improvement in response variables with a combined supplementation of enzymes was not synergistic because supplementation of individual enzymes also provided strong improvements. Although crossover effects of individual enzymes on a variable might be desirable, a true interaction between enzymes in improving the measured response variables was prevented.

6.4 Conclusions and Future Research

In North America and particularly in Western Canada, the production of biofuel is expected to continue to rise significantly over the next few years. Generally, biofuel can be defined as solid, liquid or gas fuel consisting of, or derived from biomass. Biofuel includes biodiesel that is derived from oil seeds or ethanol that is derived from grain, particularly corn and to a less extent wheat. The latter results in corn or wheat DDGS as a by-product. Relative to current production, an additional production of 5 billion gallons of ethanol and 1 billion gallons of biodiesel is predicted in North America in the next five years. The increase will require more wheat and corn to meet the demand for biofule industry. The resulting competition for grain among users may enhance production of these grains to about 35 to 40 million tonnes above current production. This increase will however not be enough to meet demands and will ultimately result in continued high prices for grain due to excess demand. Indeed, the price of wheat has risen by 75% within the last two years and the price of corn has doubled within the last

year. Ten million more tonnes of oilseeds are also expected to be produced to meet the demands of the biofuel industry.

In Western Canada, the supply of both canola meal and DDGS is predicted to increase by 2 to 3 million tonnes. Although this supply will be enough to meet local demand for protein feedstuffs by local farmers, feed grain prices prices will increase resulting in higher production costs for swine farmers, unless the stockpiles of DDGS and protein meals that result from the biofuel plants can be effectively used for animal feed. To make the switch, the digestible nutrient contents of these by-products must be characterized and enhanced using further processing. Enhanced nutrient digestibility is particularly important for monogastrics such as swine that do not have the necessary enzymes needed to digest high-fibre feedstuffs.

This thesis demonstrated that wheat millrun, which is a result of the dry milling of wheat into flour, can be processed into an effective feedstuff for grower-finisher and weaned pigs through enzyme addition. Arabinoxylans and phytate that are present in wheat millrun; and their anti-nutritive effects can be reduced by supplementing diets containing wheat millrun with xylanase and phytase. The by-product fractions contained in millrun determined the degree of response to enzyme addition meaning that more work is needed to identify substrate profiles for individual by-products to tailor specific enzymes to degrade them. An *in-vitro* digestibility technique is another route that could be used as a quick guide in determining the DE content prior to diet formulation. Portal vein catheterization of pigs would be an ideal and more direct way to measure nutrient flow from the small intestine into the blood stream.

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APPENDIX A

Chemical composition of wheat by-products (% DM basis) samples collected from Western Canada

By-product	Location	Ash	CP	ADF	NDF	Ca	P
Millrun (re-ground pellets)[1]	Saskatoon, SK	6.15	17.7	19.5	41.7	0.20	0.84
Millrun	Lethbridge, AB[2]	5.50	19.6	13.6	42.0	0.10	1.16
Millrun	Lethbridge, AB	5.91	19.1	13.4	42.4	0.10	1.26
Millrun (pelleted)	Regina, SK	5.75	17.8	16.4	39.6	0.19	0.83
Millrun (pelleted)	Edmonton, AB	5.26	20.0	12.9	38.9	0.11	1.04
Middlings	Humbolt, SK	3.98	16.1	12.5	38.5	0.09	.82
Middlings	Saskatoon, SK	5.69	19.2	12.3	39.4	0.10	1.22
Middlings	Saskatoon, SK	4.24	18.4	11.8	35.2	0.09	0.83
Middlings	Saskatoon, SK	3.44	17.5	9.8	32.7	0.08	0.69
Middlings	Saskatoon, SK	5.69	19.2	12.3	39.4	0.10	1.22
Middlings	Saskatoon, SK	4.24	18.4	11.8	35.2	0.09	0.83
Middlings	Saskatoon, SK	3.44	17.5	9.8	32.7	0.08	0.69
Shorts	Humbolt, SK	5.44	19.4	12.4	36.7	0.09	1.19
Shorts (select)	Saskatoon, SK	2.44	17.4	6.0	19.6	0.06	0.52
Shorts (pelleted)	Saskatoon, SK	4.62	17.3	15.8	41.0	0.09	0.79

[1]Millrun sample used for the present study.

[2]All samples were obtained from different locations.

APPENDIX B

Effect of xylanase and phytase on ileal digestibility of non-starch polysaccharides in weaned pigs fed specific nutrient reduced diets, %

Non-starch polysaccharide	PCON	NCON	XYL	PHY	XYL +PHY
Arabinose					
Insoluble	30.6	51.06	30.53	48.91	27.87
Soluble	-123.1	-162.19	-34.94	-231.62	-4.94
Total	9.80	22.83	14.87	25.67	21.46
Xylose					
Insoluble		47.88	28.66	48.39	28.42
Soluble		-170.44	-72.57	-335.18	-53.79
Total		22.82	12.41	25.32	15.02
Total NSP					
Insoluble	23.38	48.53	30.31	48.23	31.60
Soluble	-96.3	-135.38	-34.11	-234.59	-0.61
Total	6.67	24.57	17.52	28.48	25.53
Number of observations	2	2	4	3	2